

ALASKAN KING CRAB: BERING SEA DISTRIBUTIONS AND A PARASITIC
CASTRATOR

By

Leah Sloan Zacher, B.S., M.S.

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APPROVED:

Dr. Sarah Hardy, Committee Chair

Dr. Ginny Eckert, Committee Member

Dr. Gordon Kruse, Committee Member

Dr. Lara Horstmann, Committee Member

Dr. Frank Morado, Committee Member

Dr. Matthew Wooller, Chair

Department of Marine Biology

Dr. S. Bradley Moran, Dean

College of Fisheries and Ocean Sciences

Dr. Michael Castellini, *Dean of the Graduate School*

ABSTRACT

King crab play an integral role in both marine ecosystems and fisheries; they influence benthic community structure through predation, help regulate trophic cascades, and are an important food source for large fishes, marine mammals, and humans. To sustainably manage king crab fisheries in a changing climate, it is essential to have a thorough understanding of king crab biology and behavior, as well as knowledge on how they utilize and interact with other components of the ecosystem. I investigated factors important to king crab sustainability and management, including distribution patterns and a parasitic castrator.

Rhizocephalan barnacles in the genus *Briarosaccus* parasitize and castrate king crab hosts, thereby preventing host reproduction and potentially altering host abundance. In Alaska, prevalence is generally low (< 1% infection rate), yet higher prevalence has occurred in localized bays and fjords. I studied the larval biology of *Briarosaccus regalis* infecting *Paralithodes camtschaticus* (red king crab) to better understand how environmental factors in Alaska may influence prevalence. Maximum larval *B. regalis* survival occurred from 4 to 12 °C and at salinities between 25 and 34. Given these parameters, current conditions in the Gulf of Alaska and Bering Sea appear favorable for high survival of *B. regalis* larvae. Rhizocephalans not only castrate their hosts, but they cause changes in host morphology, physiology, and behavior. I used an untargeted metabolomics (liquid chromatography mass spectrometry) approach to compare the metabolite profiles (e.g., signaling molecules, hormones) of *P. camtschaticus* and *Lithodes aequispinus* (golden king crab) with and without rhizocephalan infections. Hundreds of putative metabolites were identified, yet few differed with crab sex and no metabolites could differentiate infected from healthy crab (regardless of crab sex). There were large variations in

the crab metabolome with collection year and location, perhaps associated with environmental variability, which likely masked differences between sex and infection status.

Summer distributions of Bristol Bay red king crab are well documented from surveys, but their distribution patterns at other times of year are poorly understood. Daily fishing logs, kept by vessel skippers in the red king crab fleet since 2005, contain detailed information on the spatial distribution of fishery effort and catch of legal sized male crab during the autumn crab fishery. However, data contained in these hand-written logbooks have not been readily accessible. I digitized daily fishing logs from 2005 to 2016 and used spatial information to infer geographic distributions. These distributions were compared across temperature regimes. In warm years (2005, 2014 – 2016) crab aggregated in the center of Bristol Bay, while in cold years (2007 – 2013) they were closer to the Alaska Peninsula. There are regions in Bristol Bay that are closed to the bottom trawl fisheries to protect red king crab; these results have management implications because they show the extent to which crab use these closure areas in the autumn, shortly before the winter trawl fisheries begin. As temperatures continue to shift in the Bering Sea, it will be important to continue monitoring crab distributions outside the summer survey period. Overall, these studies should help guide the placement of trawl closure areas, predict crab movement with temperature changes, understand the larval biology of *B. regalis* and what that could mean with climate change, and lead to a better understanding of the physiology of *Briarosaccus* infection.

DEDICATION

To my parents, for teaching me the joys of nature

From the graceful beauty of Calochortus, to the dance of Flabellinopsis

From tiny gemmae cups, to a monarch emerging from a chrysalis

You taught me to observe

Finding the wily trilobites, getting inked by an octopus

How to fool a Mimulus, how to catch a Sceloporus

You taught me to listen

What is the call of a canyon wren, what is a radiolarian

Watch a Cryptochiton run, how does a Paramecium swim

You taught me to learn

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CHAPTER 1: INTRODUCTION

As the climate changes, marine ecosystems are expected to undergo significant shifts in the physical and chemical environment, which include changes in temperature, freshwater inputs, pH, and ocean currents (Stock *et al.*, 2011; Rhein *et al.*, 2013). These abiotic factors will drive a plethora of changes in marine communities, including species abundance, distribution, phenology, and interactions (Ottersen *et al.*, 2010; Pörtner & Peck, 2010). Managing fisheries and ecosystems sustainably in the face of these changes requires flexible, adaptive management strategies that can cope with rapid change and uncertainty in all portions of an ecosystem (Johnson & Welch, 2010).

New management frameworks, including the ecosystem approach to fisheries management and ecosystem-based fisheries management, include the impacts of ecosystem processes on harvested species (Pikitch *et al.*, 2004; Long *et al.*, 2015; Skern-Mauritzen *et al.*, 2015). Under conditions of rapid environmental change, and worldwide overfishing (Jackson *et al.*, 2003; Myers & Worm, 2003), it is essential for management strategies to incorporate the connections among target species, non-target species, and the rest of the ecosystem. However, despite the potential benefits of these approaches, ecosystem processes are rarely considered in fisheries management (Skern-Mauritzen *et al.*, 2015). In particular, climate-related changes in parasitic diseases are poorly understood, but their inclusion in fishery management decisions may be essential for the sustainability of our future ecosystems and fisheries (Burge *et al.*, 2014).

Parasites are often an overlooked portion of ecosystems, but they have the potential to influence community structure and food web linkages (Bernot & Lamberti, 2008; Lafferty *et al.*, 2008; Dunne *et al.*, 2013). The structural, behavioral, and taxonomic diversity of parasites allows for a dynamic range in host-parasite relationships; parasite effects on hosts can range

from lethal (parasitoids; Gómez-Gutiérrez *et al.*, 2006) to inferring some benefits on the host, like increased salinity tolerance (Piscart *et al.*, 2007), with minor negative impacts. High parasite diversity can be an indicator of ecosystem health, although this can depend on the types of parasites present (Hudson *et al.*, 2006; Palm & Rückert, 2009). Generalist parasites can reduce biodiversity through apparent competition, whereby more resistant and abundant hosts transfer parasites to less resistant and less abundant hosts; in contrast, specialist parasites can increase biodiversity through frequency dependent parasitism, where more abundant and dense host populations will have greater parasitism (Hudson *et al.*, 2006). The response of a host population to ecosystem degradation will depend on the stressors present and the resistance of both parasite and host to those stressors (Marcogliese, 2008).

It is essential to consider the role of parasites during fisheries management (Kuris & Lafferty, 1992), especially under shifting climate regimes (Sheath *et al.*, 2016). For a fished host, like *Chionoecetes bairdi* (Tanner crab) infected with parasitic dinoflagellates (causing bitter crab disease), the parasite can influence host population sustainability and change recovery times from overfishing (Siddeek *et al.*, 2010). Temperature plays an important role in mediating interactions between parasites and their hosts (Thomas & Blanford, 2003). Temperature can affect parasitism rates by directly influencing the parasite, influencing the host (especially the host's immune system), or changing the interaction between the two (Murdock *et al.*, 2012). In general, infection rates and even parasite virulence and abundance are expected to increase with current climate change scenarios (Marcogliese, 2008). However, predicting effects of climate change on a parasite-host system is difficult because there are numerous mechanisms through which changes in climate can influence the host, parasite, and their interactions. For example, a trematode was found to cause the most limb deformities in its amphibian host at moderate

temperatures because there was a linear increase with temperature in both parasite penetration time into the host and host development rate (i.e., the time period over which the host was most vulnerable to parasite establishment) (Paull *et al.*, 2012). Detailed information is needed on how changes in climate affect different parts of the host and parasite life cycles, and the non-linear relationships that can result (Paull *et al.*, 2012).

Research Objectives

My dissertation was conducted as part of a National Science Foundation (NSF) Integrative Graduate Education and Research Traineeship (IGERT) in Marine Ecosystem Sustainability in the Arctic and Subarctic (MESAS). As such, my work uses diverse techniques and integrates data from different disciplines in the social and natural sciences, with the ultimate goal of promoting sustainable king crab populations and fisheries. **Chapters 2 and 3** describe research involving the poorly studied king crab parasite, *Briarosaccus*. This parasite castrates its king crab hosts rendering them unable to reproduce, and at high prevalence could therefore have detrimental effects on crab populations. I examined aspects of the parasite's biology that could help predict and manage changes in prevalence, including larval tolerance under different environmental conditions (chapter 2) and the physiological changes in metabolites of infected king crab (chapter 3). In **chapter 4** I use data from fishermen's logbooks to examine red king crab (*Paralithodes camtschaticus*) distribution patterns in Bristol Bay, Alaska and how they change with temperature regimes. The significance of this work in the context of fisheries and climate is discussed in the conclusion (**chapter 5**), along with directions for future work.

King Crab in Alaska

In Alaska, three species of large lithodid king crab are commercially harvested: the red (*Paralithodes camtschaticus*), blue (*Paralithodes platypus*), and golden (*Lithodes aequispinus*) king crab. These crab species are native to the subarctic waters of the North Pacific; red and blue king crab primarily inhabit areas on the shallow continental shelves, while golden king crab are found in deeper waters on the continental slope (Stevens & Lovrich, 2014). As large benthic predators, these crab influence benthic community structure through predation, help regulate trophic cascades, and are an important food source for large fishes, marine mammals, and humans (Boudreau & Worm, 2012). The exploitation of Alaskan king crab in commercial fisheries has a long history beginning in the 1920s (Gray *et al.*, 1965; Blackford, 1979). King crab populations have fluctuated dramatically, leading to boom and bust fisheries (Kruse *et al.*, 2010). Large population crashes were likely caused by multiple factors, including overfishing and environmental change (Orensanz *et al.*, 1998; Zheng & Kruse, 2006; Bechtol & Kruse, 2009). Bristol Bay red king crab have recovered to smaller yet sustainable stock levels (Kruse *et al.*, 2010) and are currently the largest king crab fishery in Alaska, with an ex-vessel value ranging over \$62 - 117 million annually between 2005 and 2014 (Garber-Yonts & Lee, 2016).

In Alaska, the Aleutian Islands and Bering Sea king crab fisheries are co-managed by the Alaska Department of Fish and Game (ADF&G) and the National Marine Fisheries Service (NMFS), with ADF&G responsible for day-to-day management under the guidance of the federal king and Tanner crab fishery management plan (Bush *et al.*, 2016). Nearshore (0-3 NM) fisheries in other regions of Alaska are managed solely by the state (ADF&G, 2014), while fisheries further offshore (3-200 NM) are federally managed. Although management strategies differ among stocks, and with the amount of data available, many Alaska crab fisheries are

managed under management plans with single-species stock assessment models or harvest strategies (ADF&G, 2014; Bush *et al.*, 2016). However, Alaska fisheries management approaches do generally take many ecosystem components into consideration. Marine protected areas limit or exclude fishing to preserve ecosystem structure and function, benthic habitats, and vulnerable species (Ackley & Witherell, 1999; Witherell & Woodby, 2005). The Magnuson-Stevens Fishery Conservation and Management Act requires that all United States federal fisheries define and protect essential fish habitat (NOAA, 2007). The North Pacific Fishery Management Council (management of Alaska's federal fisheries) has an ecosystem committee, which advises the Council on ecosystem issues and is working to increase ecosystem-based management in Alaska (Council motion, February 11, 2013). In addition, there is an ecosystem section in the stock assessment reports (Bush *et al.*, 2016).

Parasitic Barnacles

Parasitic (rhizocephalan) barnacles of the genus *Briarosaccus* can infect all commercially-harvested Alaskan king crab species, with *B. regalis* infecting red and blue king crab and *B. auratum* infecting golden king crab (previously both *B. callosus*, Noever *et al.*, 2016). Rhizocephalan barnacles are parasitic castrators that primarily infect decapod crustaceans and are only morphologically recognizable as barnacles by their larval stages (Høeg, 1995). As adults, rhizocephalans are primarily internal parasites; their body form is highly simplified, consisting of branching rootlets (the interna; Fig. 1.1A) that spread throughout the internal organs of the host (Bresciani & Høeg, 2001). When the rhizocephalan is ready to reproduce, a primordium that lies just beneath the soft abdomen cuticle, gives rise to a sac-like externa that erupts through the abdomen of the host (Fig. 1.1B). Infected crab raise and care for the parasite's larvae as they develop in the externa. Male and female crab groom the externa as if it

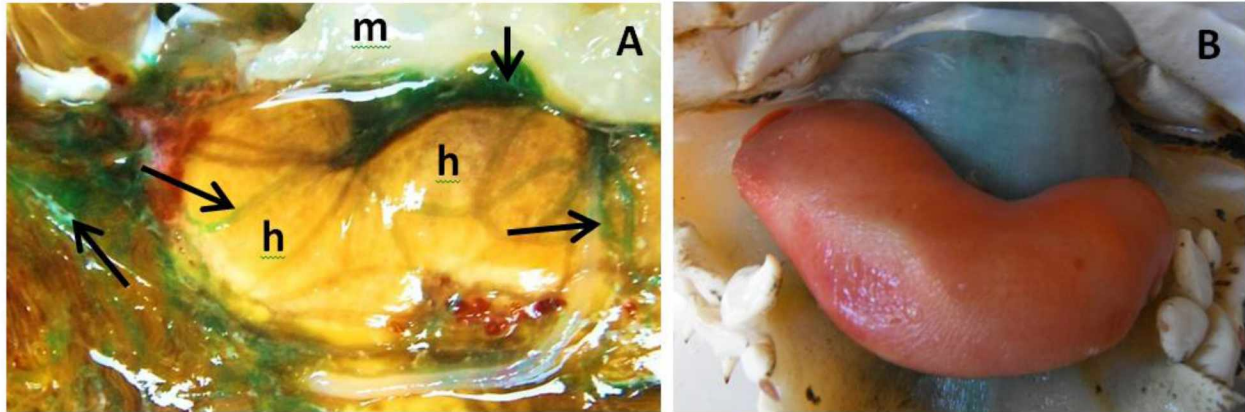


Figure 1.1. A) Crab viscera (h, hepatopancreas; m, muscle) with rhizocephalan interna (green strings at arrows) and B) rhizocephalan externa (egg sac) under a king crab's abdomen.

was their own egg mass, and pump their abdomens to assist with parasite larval release into the water column (Ritchie & Høeg, 1981; deVries *et al.*, 1989).

While prevalence and distribution of *Briarosaccus* in Alaska is poorly quantified, records generally indicate a low-level prevalence ($< 1\%$ of crabs infected) throughout king crab populations (unpublished ADF&G survey and observer data). However, outbreaks have occurred where over half of the king crab population was infected and castrated, such as around Kodiak Island in 1969 when 67% of golden king crab were infected (McMullen & Yoshihara, 1970) and in some areas of southeast Alaska in 1984 when 76% of blue king crab were infected (Hawkes *et al.*, 1986). Outbreaks of this magnitude would greatly reduce reproductive output in king crab populations and could lead to population declines. It is essential that we understand the biology of *Briarosaccus* and the factors that influence prevalence in king crab populations, especially as environmental conditions shift with climate change.

In Summary

This work aims to increase our understanding of different environmental components that affect king crab in Alaska, and the fisheries that they support. The work described in the next

three chapters has the following aims: 1) increase our knowledge of *Briarosaccus* larval biology and survival, with the ultimate goal of understanding how climate change will influence parasite prevalence, 2) lay the foundation for experiments on how *Briarosaccus* can manipulate and control its hosts by examining how metabolites differ between infected and non-infected crab; these experiments could help in future biomarker development for rapid assessment of infection prevalence, and 3) determine how data collected and recorded by fishermen could be used to fill data gaps in our understanding of king crab distribution patterns under changing temperature regimes. Ideally these distribution patterns will be used by managers to design management boundaries to protect king crab.

References

- Ackley, D. and Witherell, D. 1999. Development of a marine habitat protection area in Bristol Bay, Alaska. In: *Lowell Wakefield Fisheries Symposium. Ecosystem Approaches for Fisheries Management*. 1998 Sept 30 - Oct 3; Anchorage, Alaska, pp. 511–526.
- University of Alaska Sea Grant College Program (AK-SG-99-01), Fairbanks, Alaska
- ADF&G. 2014. *2014-2015 Commercial King and Tanner Crab Fishing Regulations*. Alaska Department of Fish and Game, Juneau, Alaska.
- Bechtol, W.R. and Kruse, G.H. 2009. Reconstruction of historical abundance and recruitment of red king crab during 1960-2004 around Kodiak, Alaska. *Fisheries Research*, **100**: 86–98.
- Bernot, R.J. and Lamberti, G.A. 2008. Indirect effects of a parasite on a benthic community: An experiment with trematodes, snails and periphyton. *Freshwater Biology*, **53**: 322–329.
- Blackford, M.G. 1979. Pioneering a modern small business: Wakefield Seafoods and the Alaskan frontier. In: *Industrial Development and the Social Fabric, Vol. 6*. Emerald Group Publishing Limited, Bingley.

- Boudreau, S.A. and Worm, B. 2012. Ecological role of large benthic decapods in marine ecosystems: A review. *Marine Ecology Progress Series*, **469**: 195–213.
- Bresciani, J. and Høeg, J.T. 2001. Comparative ultrastructure of the root system in rhizocephalan barnacles (Crustacea: Cirripedia: Rhizocephala). *Journal of Morphology*, **249**: 9–42.
- Burge, C.A., Mark Eakin, C., Friedman, C.S., Froelich, B., Hershberger, P.K., Hofmann, E.E., Petes, L.E., Prager, K.C., Weil, E., Willis, B.L., Ford, S.E. and Harvell, C.D. 2014. Climate change influences on marine infectious disease: Implications for management and society. *Annual Review of Marine Science*, **6**: 249–277.
- Bush, K., Dorn, M., Eckert, G., Foy, R., Garber-Yonts, B., Hamazaki, T., Ianelli, J., Punt, A., Rugolo, L., Siddeek, M., Stockhausen, W., Slater, L., Stram, D., Szuwalski, C., Turnock, B., Weber, D. and Zheng, J. 2016. *Stock Assessment and Fishery Evaluation Report for the King and Tanner Crab Fisheries of the Bering Sea and Aleutian Islands Regions*. North Pacific Fishery Management Council. Anchorage, Alaska.
- deVries, M., Rittschof, D. and Forward, R. 1989. Response by rhizocephalan-parasitized crabs to analogues of crab larval-release pheromones. *Journal of Crustacean Biology*, **9**: 517–524.
- Dunne, J.A., Lafferty, K.D., Dobson, A.P., Hechinger, R.F., Kuris, A.M., Martinez, N.D., McLaughlin, J.P., Mouritsen, K.N., Poulin, R., Reise, K., Stouffer, D.B., Thieltges, D.W., Williams, R.J. and Zander, C.D. 2013. Parasites affect food web structure primarily through increased diversity and complexity. *PLOS Biology*, **11**: 1–17.
- Garber-Yonts, B. and Lee, J. 2016. *Economic Status Report Summary: BSAI Crab Fisheries, 2016*. North Pacific Fishery Management Council. Seattle, Washington.

- Gómez-Gutiérrez, J., Peterson, W.T. and Morado, J.F. 2006. Discovery of a ciliate parasitoid of euphausiids off Oregon, USA: *Collinia oregonensis* n. sp. (Apostomatida: Colliniidae). *Diseases of Aquatic Organisms*, **71**: 33–49.
- Gray, G.W., Roys, R., Simon, R.J. and Lall, D.F. 1965. *Development of the king crab fishery off Kodiak Island*. Alaska Department of Fish and Game, Information Leaflet 52.
- Hawkes, C.R., Meyers, T.R. and Shirley, T.C. 1986. Prevalence of the parasitic barnacle *Briarosaccus callosus* on king crabs of southeastern Alaska. *Transactions of the American Fisheries Society*, **115**: 252–257.
- Høeg, J.T. 1995. The biology and the life-cycle of the rhizocephala (Cirripedia). *Journal of the Marine Biological Association of the United Kingdom*, **75**: 517–550.
- Hudson, P.J., Dobson, A.P. and Lafferty, K.D. 2006. Is a healthy ecosystem one that is rich in parasites? *Trends in Ecology and Evolution*, **21**: 381–385.
- Jackson, J.B.C., Kirby, M.X., Berger, W.H., Bjorndal, K.A., Botsford, L.W., Bourque, B.J., Bradbury, R.H., Cooke, R., Erlandson, J., Estes, J.A., Hughes, T.P., Kidwell, S., Lange, C.B., Lenihan, H.S., Pandolfi, J.M., Peterson, C.H., Steneck, R.S., Tegner, M.J. and Warner, R.R. 2003. Historical overfishing and the recent collapse of coastal ecosystems. *Science*, **293**: 629–637.
- Johnson, J.E. and Welch, D.J. 2010. Marine fisheries management in a changing climate: A review of vulnerability and future options. *Reviews in Fisheries Science*, **18**: 106–124.
- Kruse, G.H., Zheng, J. and Stram, D.L. 2010. Recovery of the Bristol Bay stock of red king crabs under a rebuilding plan. *ICES Journal of Marine Science*, **67**: 1866–1874.
- Kuris, A.M. and Lafferty, K.D. 1992. Modelling crustacean fisheries: Effects of parasites on management strategies. *Canadian Journal of Fisheries and Aquatic Sciences*, **49**: 327–336.

- Lafferty, K.D., Allesina, S., Arim, M., Briggs, C.J., De Leo, G., Dobson, A.P., Dunne, J.A., Johnson, P.T.J., Kuris, A.M., Marcogliese, D.J., Martinez, N.D., Memmott, J., Marquet, P.A., McLaughlin, J.P., Mordecai, E.A., Pascual, M., Poulin, R. and Thielctges, D.W. 2008. Parasites in food webs: The ultimate missing links. *Ecology Letters*, **11**: 533–546.
- Long, R.D., Charles, A. and Stephenson, R.L. 2015. Key principles of marine ecosystem-based management. *Marine Policy*, **57**: 53–60.
- Marcogliese, D.J. 2008. The impact of climate change on the parasites and infectious diseases of aquatic animals. *Revue Scientifique et Technique (International Office of Epizootics)*, **27**: 467–84.
- McMullen, J. and Yoshihara, H. 1970. An incidence of parasitism of deepwater king crab, *Lithodes aequispina*, by the barnacle *Briarosaccus callosus*. *Journal of the Fisheries Research Board of Canada*, **27**: 818–821.
- Murdock, C.C., Paaijmans, K.P., Bell, A.S., King, J.G., Hillyer, J.F., Read, A.F. and Thomas, M.B. 2012. Complex effects of temperature on mosquito immune function. *Proceedings of the Royal Society B: Biological Sciences*, **279**: 3357–3366.
- Myers, R.A. and Worm, B. 2003. Rapid worldwide depletion of predatory fish communities. *Nature*, **423**: 280–283.
- NOAA. 2007. *Magnuson-Stevens Fishery Conservation and Management Act. Public Law 94-265 as amended by the Magnuson-Stevens Fishery Conservation and Management Reauthorization Act (P.L. 109-479)*. National Oceanic and Atmospheric Administration, Silver Spring, MD.

- Noever, C., Olson, A. and Glenner, H. 2016. Two new cryptic and sympatric species of the king crab parasite *Briarosaccus* (Cirripedia: Rhizocephala) in the North Pacific. *Zoological Journal of the Linnean Society*, **176**: 3–14.
- Orensanz, J.M., Armstrong, J., Armstrong, D. and Hilborn, R. 1998. Crustacean resources are vulnerable to serial depletion – the multifaceted decline of crab and shrimp fisheries in the greater Gulf of Alaska. *Reviews in Fish Biology and Fisheries*, **176**: 117–176.
- Ottersen, G., Kim, S., Huse, G., Polovina, J.J. and Stenseth, N.C. 2010. Major pathways by which climate may force marine fish populations. *Journal of Marine Systems*, **79**: 343–360.
- Palm, H.W. and Rückert, S. 2009. A new approach to visualize ecosystem health by using parasites. *Parasitology Research*, **105**: 539–553.
- Paull, S.H., LaFonte, B.E. and Johnson, P.T.J. 2012. Temperature-driven shifts in a host-parasite interaction drive nonlinear changes in disease risk. *Global Change Biology*, **18**: 3558–3567.
- Pikitch, E.K., Santora, C., Babcock, E.A., Bakun, A., Bonfil, R., Conover, D.O., Dayton, P., Doukakis, P., Fluharty, D., Heneman, B., Houde, E.D., Link, J., Livingston, P.A., Mangel, M., McAllister, M.K., Pope, J. and Sainsbury, K.J. 2004. Ecosystem-based fishery management. *Science*, **305**: 346–347.
- Piscart, C., Webb, D. and Beisel, J.N. 2007. An acanthocephalan parasite increases the salinity tolerance of the freshwater amphipod *Gammarus roeseli* (Crustacea: Gammaridae). *Die Naturwissenschaften*, **94**: 741–747.
- Pörtner, H.O. and Peck, M.A. 2010. Climate change effects on fishes and fisheries: Towards a cause-and-effect understanding. *Journal of Fish Biology*, **77**: 1745–1779.

- Rhein, M., Rintoul, S., Aoki, S., Campos, E., Chambers, D., Feely, R.A., Gulev, S., Johnson, G.C., Josey, S.A., Kostianoy, A., Mauritzen, C., Roemmich, D., Talley, L.D. and Wang, F. 2013. Observations: Ocean. In: *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (T. F. Stocker et al., eds), pp. 255–315. Cambridge University Press, New York.
- Ritchie, L. and Høeg, J.T. 1981. The life history of *Lernaodiscus porcellanae* (Cirripedia: Rhizocephala) and co-evolution with its porcellanid hosts. *Journal of Crustacean Biology*, **1**: 334–347.
- Sheath, D.J., Andreou, D. and Britton, J.R. 2016. Interactions of warming and exposure affect susceptibility to parasite infection in a temperate fish species. *Parasitology*, **143**: 1340–1346.
- Siddeek, M.S.M., Zheng, J., Morado, J.F., Kruse, G.H. and Bechtol, W.R. 2010. Effect of bitter crab disease on rebuilding in Alaska Tanner crab stocks. *ICES Journal of Marine Science*, **67**: 2027–2032.
- Skern-Mauritzen, M., Ottersen, G., Handegard, N.O., Huse, G., Dingsør, G.E., Stenseth, N.C. and Kjesbu, O.S. 2015. Ecosystem processes are rarely included in tactical fisheries management. *Fish and Fisheries*, **17**: 165–175.
- Stevens, B.G. and Lovrich, G.A. 2014. Chapter 1: King crabs of the world: species and distributions. In: *King Crabs of the World, Biology and Fisheries Management* (B. G. Stevens, ed), pp. 1–30. CRC Press, Boca Raton.

- Stock, C.A., Alexander, M.A., Bond, N.A., Brander, K.M., Cheung, W.W.L., Curchitser, E.N., Delworth, T.L., Dunne, J.P., Griffies, S.M., Haltuch, M.A., Hare, J.A., Hollowed, A.B., Lehodey, P., Levin, S.A., Link, J.S., Rose, K.A., Rykaczewski, R.R., Sarmiento, J.L., Stouffer, R.J., Schwing, F.B., Vecchi, G.A. and Werner, F.E. 2011. On the use of IPCC-class models to assess the impact of climate on Living Marine Resources. *Progress in Oceanography*, **88**: 1–27.
- Thomas, M.B. and Blanford, S. 2003. Thermal biology in insect-parasite interactions. *Trends in Ecology and Evolution*, **18**: 344–350.
- Witherell, D. and Woodby, D. 2005. Application of marine protected areas for sustainable production and marine biodiversity off Alaska. *Marine Fisheries Review*, **67**: 1–28.
- Zheng, J. and Kruse, G.H. 2006. Recruitment variation of eastern Bering Sea crabs: climate-forcing or top-down effects? *Progress in Oceanography*, **68**: 184–204.

CHAPTER 2: LARVAL BIOLOGY AND ENVIRONMENTAL TOLERANCES OF THE KING CRAB PARASITE *BRIAROSACCUS REGALIS*¹

Abstract

Rhizocephalan barnacles in the genus *Briarosaccus* parasitize and castrate king crab hosts, thereby preventing host reproduction and potentially altering host abundance. To better understand how environmental factors in Alaska may influence *Briarosaccus* prevalence, we studied the effects of temperature and salinity on the larvae of *Briarosaccus regalis* (previously *B. callosus*). Nauplius larvae were reared at 7 temperatures (2 to 16 C) and 8 salinities (19 to 40) to determine larval survival and development rates. Maximum survival occurred from 4 to 12 C and at salinities between 25 and 34. In the Gulf of Alaska and Bering Sea, ocean temperatures and salinities are often within these ranges, thus current conditions appear favorable for high *B. regalis* larval survival. In addition, temperature was negatively correlated with larval development time, thus warmer waters can reduce the time larvae are exposed to the dangers of the planktonic environment. Since only female *B. regalis* larvae can infect crabs, we investigated the sex ratios of *B. regalis* broods at different temperatures and how size and morphological traits can be used to sex cyprid larvae. Larval rearing temperature did not affect brood sex ratio ($F = 0.947$, $P = 0.369$), but sex ratio varied among broods ($F = 221.9$; $P < 0.001$). Male larvae ($424.5 \pm 24.3 \mu\text{m}$ (mean \pm 1SD)) were significantly larger than female larvae ($387.6 \pm 22.7 \mu\text{m}$ (mean \pm 1SD); $F = 1,221.4$; $P < 0.001$), consistent with other rhizocephalan cyprids, but sizes overlapped between the sexes such that morphological traits were also necessary for

¹ Sloan, L.M. and S.M. Hardy. 2017. Larval biology and environmental tolerances of the king crab parasite *Briarosaccus regalis*. *Journal of Parasitology* 103: 22-31.

determining sex. Overall, this study provides new information on the larval biology, larval morphology, and environmental tolerances of *B. regalis*, an important king crab parasite.

Introduction

The distribution and prevalence of parasites is closely tied to environmental factors (Guernier *et al.*, 2004; Sheehan *et al.*, 2011). In particular, temperature plays an important role in mediating the interactions between parasites and their hosts (Thomas & Blanford, 2003). Temperature can affect parasite prevalence and abundance by directly influencing the parasite or host (e.g., changing the physiology, fecundity, growth rates, immune response, or host recovery time), or altering the interaction between the two (Blanford & Thomas, 2000; Lazzaro *et al.*, 2008; Murdock *et al.*, 2012). Because parasites are often restricted by cold water temperatures (Harvell *et al.*, 2002), warming can cause outbreaks in areas of previously low abundance. For instance, in Long Island Sound (USA), large-scale die-offs of *Homarus americanus* (American lobster) occurred when parasitic amoeba outbreaks coincided with unusually warm water temperatures (Pearce & Balcom, 2005). Understanding how environmental factors like temperature influence the host-parasite relationship can help explain parasite distributions and prevalence in host populations. In this study, we focused on temperature and salinity effects on the larval phase of the rhizocephalan barnacle *Briarosaccus regalis*, infecting the host *Paralithodes camtschaticus* (red king crab).

Paralithodes camtschaticus is a subarctic lithodid crab that primarily inhabits continental shelves around the North Pacific (Stevens & Lovrich, 2014). This species was also introduced to the Barents Sea, where the population is rapidly expanding (Oug *et al.*, 2011). As a large-bodied crustacean, *P. camtschaticus* plays an integral role in both the marine ecosystem and fisheries (e.g., Kruse *et al.*, 2010; Boudreau & Worm, 2012). Large crustaceans influence benthic

community structure through predation, help regulate trophic cascades, and are an important food source for large fish, marine mammals, and humans (Boudreau & Worm, 2012). Very little is known about king crab parasites or their effects on fisheries, although several microbial, protistan, helminthic, and crustacean parasites that infect *P. camtschaticus* have been identified (Morado, 2011; Morado *et al.*, 2014).

Rhizocephalan barnacles are parasitic castrators that primarily infect decapod crustaceans and are only morphologically recognizable as barnacles by their nauplius and cyprid larval stages (Høeg, 1995). As adults, rhizocephalans are primarily internal parasites, with an external reproductive structure called the externa (Bresciani & Høeg, 2001). Female larvae infect hosts by injecting cells that divide to form a rootlet system (interna), which spreads throughout the host's body while absorbing nutrients (Høeg, 1995). When the interna is ready to reproduce, it forms the egg sac-like externa under the abdomen of the host. Male cyprids are attracted to the newly emerged externa, which they enter, metamorphose into dwarf males, and produce sperm to fertilize the eggs (Høeg, 1987).

Members of the genus *Briarosaccus* infect many lithodid species throughout the world (e.g., Hawkes *et al.*, 1986; Abello & Macpherson, 1992; Guzman *et al.*, 2002). Originally all *Briarosaccus* sp. infecting lithodid crabs were identified as *Briarosaccus callosus*, but new cryptic species of *Briarosaccus* are now recognized (Noever *et al.*, 2016). *Briarosaccus regalis* was identified on *P. camtschaticus* and *P. platypus* (blue king crab) in southeast Alaska (Noever *et al.*, 2016). However, its range likely extends across the North Pacific distribution of its hosts, throughout which *B. callosus* infections have been reported (Sloan, 1985; Hawkes *et al.*, 1986; Sparks & Morado, 1986; Isaeva *et al.*, 2005).

Effects of *Briarosaccus* on commercial king crab stocks are poorly understood (Kuris & Lafferty, 1992; Basson, 1994). However, outbreaks of other rhizocephalans have caused economic losses in other crab stocks, such as the *Callinectes sapidus* (blue crab) in the Gulf of Mexico (Lázaro-Chávez *et al.*, 1996). Population models (Negovetich & Esch, 2008) and field studies (Lafferty, 1993; Fredensborg *et al.*, 2005) suggest that parasitic castrators can reduce the density of host populations by affecting fecundity, and the magnitude of this reduction is largely a function of parasite prevalence. Prevalence of *B. regalis* generally appears low in *P. camtschaticus* (<1%; Sloan, 1985; Hawkes *et al.*, 1986; unpubl. ADF&G survey and observer program data), although a higher prevalence has occurred in *P. platypus*, especially in isolated fjords and bays (e.g., 76% infected in Glacier Bay in 1984; Hawkes *et al.*, 1986). If the prevalence of *B. regalis* were to increase in *P. camtschaticus*, it could lower king crab fecundity, with the potential for future stock declines.

Causes of variability in rhizocephalan prevalence are diverse and range from characteristics of the host populations, like host size distribution (Sloan *et al.*, 2010) and host susceptibility (Ritchie & Høeg, 1981), to environmental characteristics, such as temperature and salinity (Reisser & Forward, 1991; Kashenko & Korn, 2002a; b, 2003). The factors that influence *B. regalis* prevalence have not been identified (Sloan, 1984; Hawkes *et al.*, 1986). In a single laboratory study, *B. regalis* larvae were successfully raised from nauplii to cyprids at 6–7 C (n = 2 broods); however, when raised at 4 C (n = 1 brood) larvae never metamorphosed into cyprids (Hawkes *et al.*, 1985), suggesting low temperatures could limit distribution. Although never tested for *B. regalis*, salinity can also limit the larval survival (Walker & Clare, 1994; Kashenko *et al.*, 2002) and distribution of rhizocephalans (Tindle *et al.*, 2004). Lower salinity caused decreased prevalence of the rhizocephalan *Loxothylacus panopaei* in populations of its

estuarine crab host, because *L. panopaei* cannot tolerate extremely low salinities (Reisser & Forward, 1991).

In order to better understand *B. regalis* prevalence and distribution in king crab populations within the North Pacific region, we investigated the effects of temperature and salinity on *B. regalis* larval survival and development rate through 4 naupliar stages to the infectious cyprid stage. In addition, because only female *B. regalis* cyprids can infect crabs, we investigated the sex ratios of *B. regalis* broods, and determined whether size and morphological traits can be used to sex cyprid larvae. In rhizocephalans, male cyprids are generally larger than females, thus cyprid length has been used as an indicator of sex in many species (Ritchie & Høeg, 1981). However, size ranges often overlap between the sexes (Walker *et al.*, 1992), and seasonal size variation can be superimposed on the sex-linked size (Høeg & Lützen, 1995). In summary, this study provides novel data on the larval biology of *Briarosaccus*, an important parasite of king crabs worldwide, and presents results of experiments on the environmental conditions under which *B. regalis* larvae can successfully develop.

Materials and Methods

Paralithodes camtschaticus with externae of the rhizocephalan *B. regalis* were collected June–July of 2013 and 2014 during leg 1 of the Alaska Department of Fish and Game (ADF&G) red king crab survey around Juneau, Alaska (southern Lynn Canal, Auke Bay, and Stephens Passage; Fig. 2.1). Average bottom temperature during each survey was 4.87 ± 0.007 C and 5.37 ± 0.008 C (mean \pm 1SE) for 2013 and 2014, respectively (ADF&G unpubl. data from temperature loggers on survey crab pots). Average bottom salinity was 32.46 ± 0.07 for 2013 and 31.64 ± 0.01 for 2014 (mean \pm 1SE; ADF&G unpubl. data from 2 CTD casts during each survey, bottom salinity averaged from the bottom 10 meters of each cast). The prevalence of red

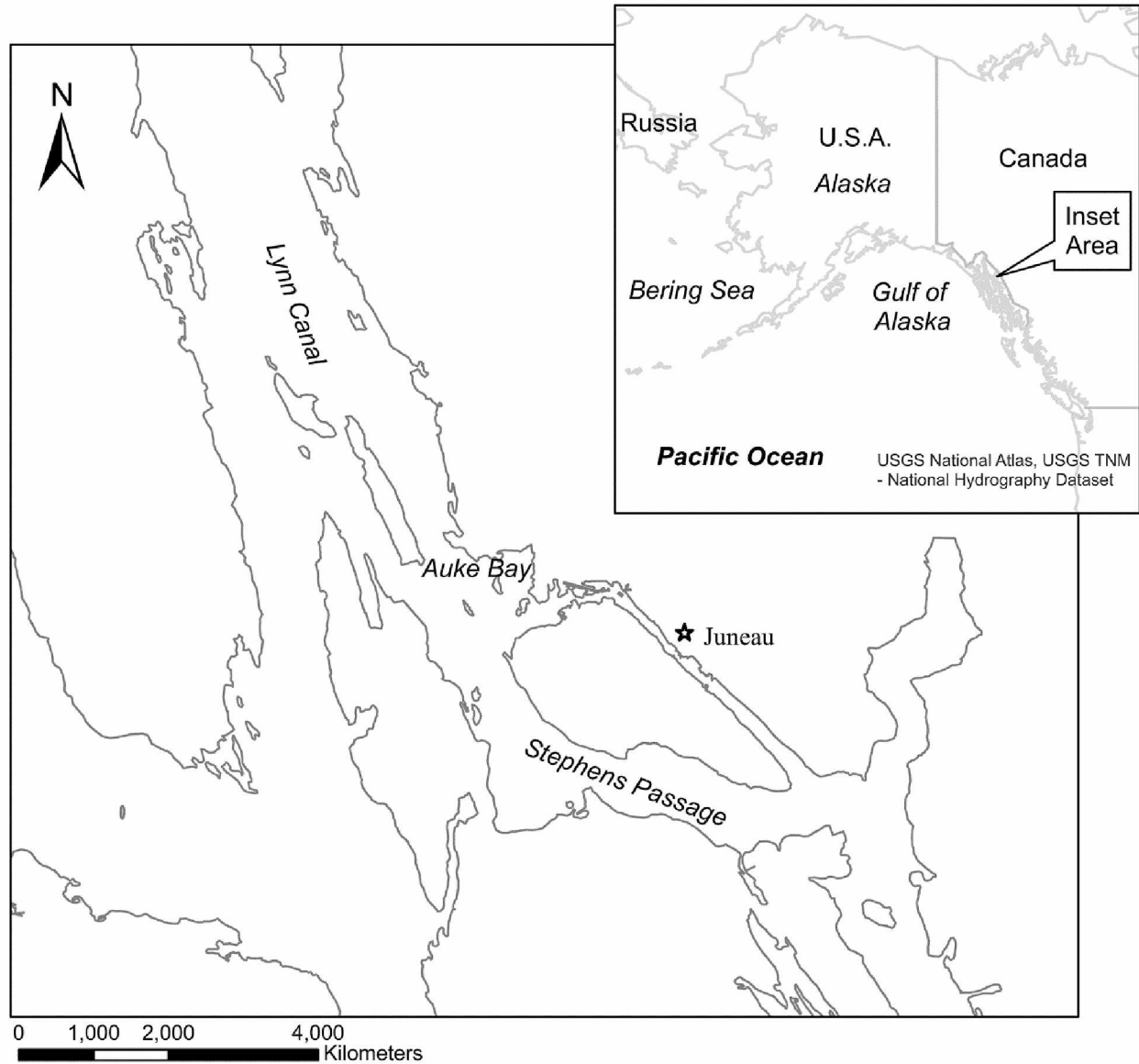


Figure 2.1. Map of collection region for *Paralithodes camtschaticus* (red king crab), near Juneau, Alaska (58.30°N, 134.42°W).

king crabs infected with *B. regalis* was 0.31% ($n = 2,273$) and 0.10% ($n = 3,968$) on leg 1 of the 2013 and 2014 surveys, respectively (ADF&G unpubl. data). All infected crabs with an external infection were collected, yielding 5 crabs on the 2013 survey and 3 on the 2014 survey. Infected crabs were transported to the Seward Marine Center of the University of Alaska Fairbanks in Seward, Alaska. Crabs were held in individual tanks in a flow-through seawater system at ambient temperature (average 8.3 C; minimum 4.6 C; maximum 13.7 C) and salinity (average 30.7;

minimum 28.2; maximum 31.8), with a light regime mimicking ambient conditions at their collection location (Juneau, Alaska). Crabs were held under laboratory conditions for up to 10 mo while multiple broods of parasite larvae were released naturally from each externa. Of the 8 infected crabs collected, 5 died in transport or never produced viable larval broods, leaving 3 crabs from which *B. regalis* larvae were collected for experiments.

To determine when *B. regalis* larvae would be released from externae, a Pasteur pipette was inserted into the mantle opening, and a few embryos were extracted and examined under a dissecting microscope. From the time when the first hatched nauplii could be observed in the externa, it took up to a week for the entire brood to hatch and be released. When hatched nauplii were first observed in the externae, crabs were placed in aquaria without flow-through water. The aquaria were equipped with an aerator and partially submerged in a tank with flow-through water to maintain ambient seawater temperature. Water changes were performed daily. Although a few nauplii were released early from the externa, the vast majority were released within a 2–3 hr period. The timing of the release had no apparent correlation with time of day. Within 12 hr of release, larvae were placed in their respective temperature and salinity treatments. The first larval molt occurred within 24 hr of hatching (Hawkes *et al.*, 1985), thus a mixture of stage I and stage II nauplii was used to initiate the temperature and salinity experiments.

Temperature and salinity experiments

Temperature treatments included 2, 4, 6, 8, 10, 12, and 16 C, while salinity treatments included 19, 22, 25, 28, 31, 34, 37, and 40. A fully crossed design was not logistically feasible. All salinity treatments were conducted at 6 C, a temperature that supports *Briarosaccus* sp. development (Hawkes *et al.*, 1985), and all temperature treatments were conducted at a salinity

of 31, which is typical of the Bering Sea where large king crab populations occur (Ladd and Stabeno, 2012), and with *P. camtschaticus* collection sites in southeast Alaska (Stone *et al.*, 1992; Ladd & Stabeno, 2012). The selection of minimum temperature and salinity values for experiments was based on preliminary trials, which demonstrated that larvae did not survive at 0 C or at a salinity of 16. Upper temperature and salinity values were selected based on extremes tolerated by other rhizocephalan species living in similar conditions (Walker & Lester, 1998; Kashenko & Korn, 2002b), with consideration of what larvae could potentially experience under natural conditions (Stone *et al.*, 1992; Stabeno *et al.*, 2007; Ladd & Stabeno, 2012). For each temperature and salinity treatment, 50 larvae from a single brood were placed into each of 5 replicate petri dishes (2.5 cm X 10 cm). The experiments were repeated across 5 separate broods, which came from the externae of 3 different host crabs (Table I).

Treatments were conducted in small refrigerators, with digital thermostats (A419; Johnson Controls, Milwaukee, Wisconsin) to control temperature within ± 0.56 C. Each refrigerator had a small fan inside to ensure that temperatures were constant throughout the chamber. All experiments were conducted with filtered (sand filter and 10 μ m filter) and UV-sterilized seawater. Dilute salinities were created by adding distilled water to the sterilized natural seawater, while high salinities were created by evaporation of seawater. Salinity and temperature measurements were made with an YSI 85 (YSI Inc., Yellow Springs, Ohio), with an accuracy of 0.1 for salinity and 0.1 C. Culture dishes were kept covered to prevent evaporation and resultant changes in salinity. To remove dead larvae and exuviae, live larvae were counted and hand pipetted into clean treatment water at 5 evenly spaced intervals throughout larval development. Since larvae raised at different temperatures developed at different rates, an approximate development time for each temperature was determined in preliminary trials and

divided by 5 to determine intervals between water changes. *Briarosaccus* larvae are lecithotrophic (Hawkes *et al.*, 1985), thus food was not provided.

Percent survival to the infectious cyprid stage was determined for all temperature and salinity treatments and average development time (days) was determined for all temperature treatments. When larvae were in the last naupliar stage, dishes were checked daily and metamorphosed cyprids were removed. When enough cyprids were available, 30 cyprids from each treatment were sexed and photographed. Maximum linear cyprid length was measured from photographs using Image J (Schneider *et al.*, 2012). Cyprids were sexed using morphological characteristics of the antennae (as in Glenner *et al.*, 1989; Moyse *et al.*, 1995). Distinguishing secondary sexual characteristics of *B. regalis* cyprids have not been previously described; we used descriptions of other members of the Peltogasteridae (Glenner *et al.*, 1989; Moyse *et al.*, 1995) and personal communication (J. T. Høeg) to describe those in *B. regalis*.

Statistical analyses

To determine temperature effects on larval survival through the cyprid stage (binomial response variable) we used a mixed effects logistic regression with the Laplace approximation (Bolker *et al.*, 2009), which produces a χ^2 test statistic. Temperature was the fixed effect and crab, brood, and dish were nested random effects, nested respectively. The same methods were also used to determine the effects of salinity on development success. Least squares linear regressions were used to determine temperature effects on the natural logarithm of larval and embryo development time. A logistic regression was used to test the effects of crab, brood nested within crab, and temperature on cyprid sex ratio, and an ANOVA was also used to determine the effects of sex, crab, and brood nested within crab on cyprid length. All post hoc pairwise comparisons were conducted with Tukey's HSD test. To visualize the underlying

distribution of cyprid lengths by larval sex and brood, we used a histogram and kernel density plots. Kernel density plots are similar to histograms, but they are smoothed using a probability density function. Where appropriate, normality and homoscedasticity were first examined using plots of the fitted values versus the residuals and Q-Q plots of the theoretical quantiles versus the standardized residuals. All statistical analyses were conducted in R (R Core Team, 2015) with $\alpha = 0.05$.

Results

Briarosaccus regalis larvae were released on average every 54 days (minimum 38, maximum 81; Table 2.1) by each parasite externa. Embryo development time was recorded as the time from one larval release to the next. Development time was determined for 7 broods and mean water temperature during development did not affect embryo development time ($F = 0.947$, $P = 0.369$, $R^2 = 0.163$; Table 2.1). Nauplii from 5 broods of larvae from 3 externae (on 3 different host crabs) were successfully reared through the infectious cyprid stage. These broods

Table 2.1. Release dates for broods of parasitic *Briarosaccus regalis* larvae from host crabs *Paralithodes camtschaticus*, embryo development time (days), mean water temperature during embryo development (time from previous larval release to release of current brood; ± 1 standard error), and sex ratios of cyprid larvae, n = sample size, n.d = no data.

Crab	Brood	Release date	Embryo development time	Embryo development water temperature (C)	% Female larvae (n)	% Male larvae (n)
1	a	19-Sep-13	59	10.50 ± 0.227	n.d.	n.d.
	b	19-Nov-13	61	10.90 ± 0.094	n.d.	n.d.
	c	8-Feb-14	81	6.70 ± 0.161	n.d.	n.d.
	d	27-Mar-14	47	5.50 ± 0.082	n.d.	n.d.
	e	4-May-14	38	5.50 ± 0.002	73.2% (41)	26.8% (15)
2	a	28-Dec-14	n.d.	$9.6 \pm 0.440^*$	92.9% (195)	7.1% (15)
	b	9-Feb-15	43	7.80 ± 0.133	30.8% (64)	69.2% (144)
3	a	15-Jan-15	n.d.	$8.90 \pm 0.369^*$	100% (210)	0% (0)
	b	2-Mar-15	46	7.10 ± 0.181	100% (210)	0% (0)

*estimated based on a development time of 54 days

will be referenced according to their crab and brood number and letter combination (Table 2.1). Crabs 1 and 3 were males, while crab 2 was a female. Crab 1 was captured in 2013, while crabs 2 and 3 were captured in 2014. Throughout the naupliar stages, larvae typically remained at or near the bottom of dishes and rarely got stuck in the surface tension, while cyprid larvae were often stuck in the surface tension.

Temperature and salinity effects

Nauplius survival through the cyprid stage differed as a function of temperature ($\chi^2 = 86.6$; $P < 0.001$). Larval survival was significantly lower at 2 and 16 C, while it was highest from 4 to 12 C (Fig. 2.2). Larval survival was highly variable among broods, especially in the extreme temperature treatments (2 C and 16 C; Fig. 2.2). Temperature and the natural logarithm of larval development time had a negative linear correlation ($R^2 = 0.972$; $F = 16,530$; $P < 0.001$).

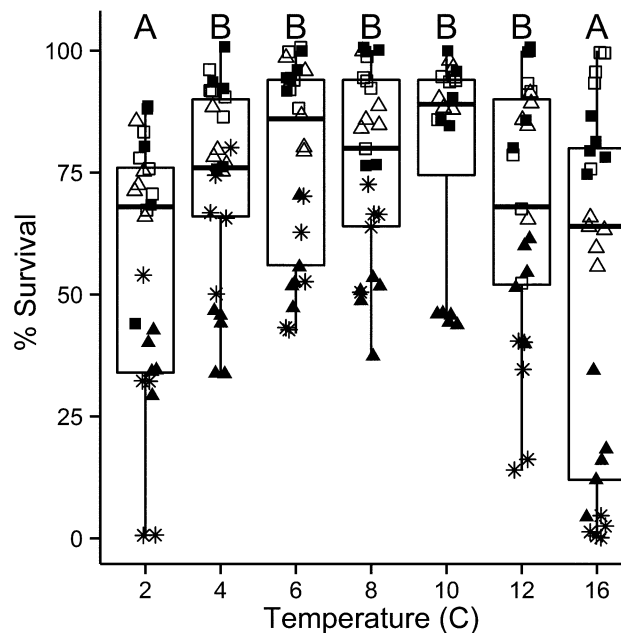


Figure 2.2. Survival through the cyprid stage for *Briarosaccus regalis* larvae under different temperature treatments. Boxplots show median, interquartile range (IQR), and whiskers that extend to the highest/ lowest value that is within 1.5 of the IQR. Percent survival for each trial dish for each crab/brood are shown within the boxplots: brood 1e = asterisk, brood 2a = closed triangle, brood 2b = open triangle, brood 3a = closed square, brood 3b = open square. Boxes not sharing a common letter have means that are statistically significantly different at $P < 0.05$.

Larval development time increased at colder temperatures, with a Q_{10} of 2.70. At 16 C development was completed in 11 days on average, while at 2 C nauplii metamorphosed into cyprids after an average of 43 days (Fig. 2.3). In addition, the variation around mean development time increased with decreasing temperature (Fig. 2.3).

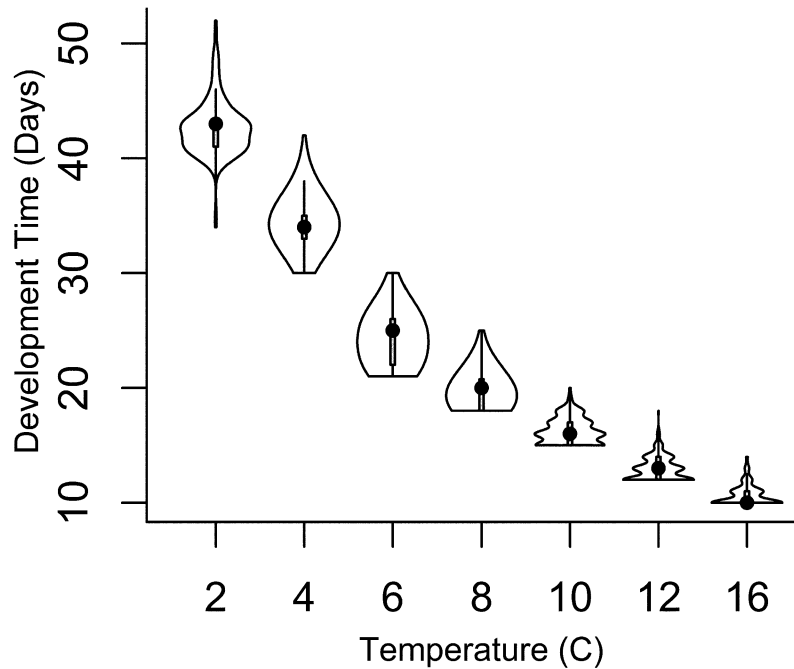


Figure 2.3. Violin plots of development time for nauplius larvae of *Briarosaccus regalis* reared at 7 different temperature regimes, with a $Q_{10} = 2.70$. Each plot shows the median (black circle), boxplot with the interquartile range (IQR; gray vertical bars) and whiskers (black vertical lines which extend to the either highest/ lowest value or $1.5 * IQR$, whichever value is closest to the median), and the kernel density plot (thin vertical curves showing frequency distribution).

Larval survival through the cyprid stage also differed as a function of salinity ($\chi^2 = 533.4$; $P < 0.001$). Maximum larval survival occurred at salinities 25 to 34, while survival dropped slightly, but significantly, at the higher salinities (37 and 40) and sharply at the lowest salinities (19 and 22; Fig. 2.4).

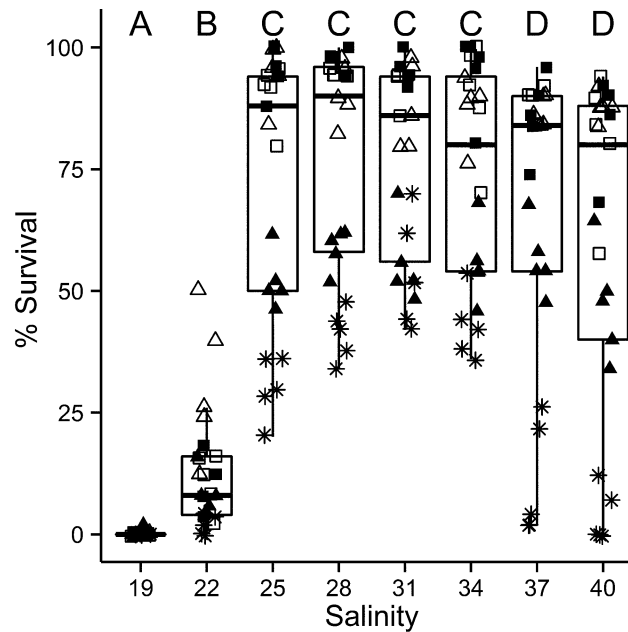


Figure 2.4. Survival through the cyprid stage for *Briarosaccus regalis* larvae under different salinity treatments. Boxplots show median, interquartile range (IQR), and whiskers that extend to the highest/ lowest value that is within 1.5 of the IQR. Percent survival for each trial dish for each crab/brood are shown within the boxplots: brood 1e = asterisk, brood 2a = closed triangle, brood 2b = open triangle, brood 3a = closed square, brood 3b = open square. Boxes not sharing a common letter have means that are statistically significantly different at $P < 0.05$.

Cyprid sex determination, sex ratios, and size

Briarosaccus regalis cyprids have not previously been sexed using morphological traits, although size was presumed to indicate sex in the only other study on the larvae of this species (Hawkes *et al.*, 1985). Sexual dimorphism of cyprid antennae (Fig. 2.5) was observed in this study based on comparisons with other members of the Peltogasteridae (Glenner *et al.*, 1989), including *Briarosaccus tenellus* (Moyse *et al.*, 1995), and by personal communication (J. T. Høeg). Male cyprids were identified by the bifurcated large aesthetasc on the third antennal segment and the enlarged subterminal aesthetasc on the fourth segment. Female larvae have 2 distinct setae in place of the large aesthetasc and a much smaller subterminal aesthetasc. In

addition, the third antennal segment of females is rounded, while this segment is more elongated in the males (Fig. 2.5).

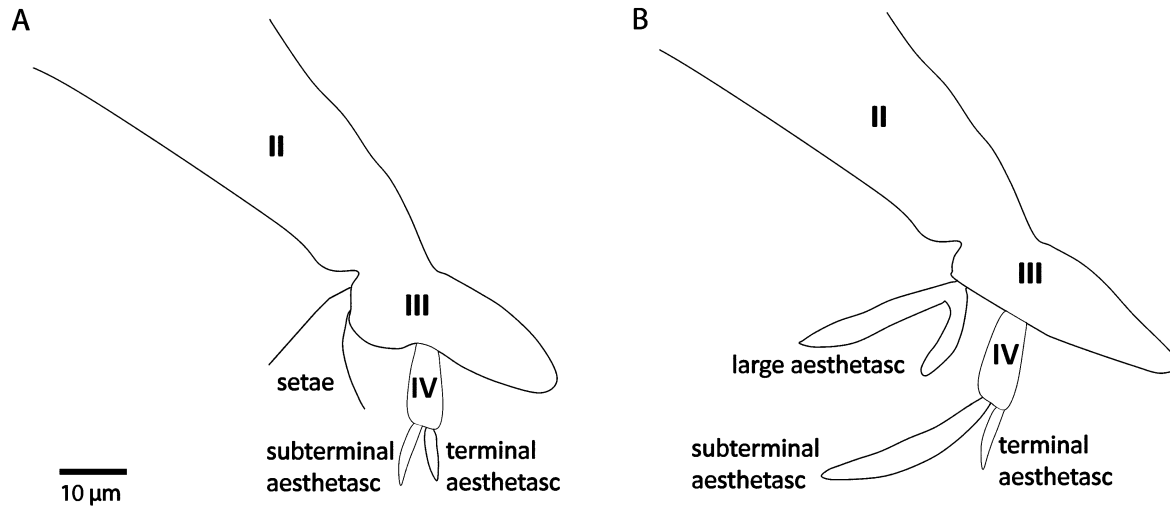


Figure 2.5. Key distinguishing characteristics on the antennae of (A) female and (B) male *Briarosaccus regalis* cyprids (modified from other Peltogastridae descriptions, e.g., Glenner *et al.*, 1989; Moyse *et al.*, 1995). Roman numerals indicate antennal segments.

Cyprid sex ratio was a function of both crab ($P < 0.001$) and brood nested within crab ($P < 0.001$), but sex ratio was not affected by larval rearing temperature ($P = 0.72$). Two broods (both from crab 3) consisted entirely of female larvae, while the other 3 broods were a mixture of male and female larvae (Table 2.1). All recorded broods were released in winter and spring (December through May), yet no pattern was apparent between sex ratio and season (e.g., larvae of both sexes were released within a few days of each other). Cyprid sex had a significant effect on larval length ($F = 1221.4$; $P < 0.001$). On average, males were larger ($424.5 \pm 24.3 \mu\text{m}$ (mean $\pm 1\text{SD}$)) than females ($387.6 \pm 22.7 \mu\text{m}$ (mean $\pm 1\text{SD}$)); however, the size distribution of the sexes substantially overlapped (Fig. 2.6). In addition, both host crab ($F = 186.3$; $P < 0.001$) and brood nested within crab ($F = 221.9$; $P < 0.001$) had significant effects on cyprid length. Larval size varied substantially in female (Fig. 2.7) and male (Fig. 2.8) larvae, both between broods

from the same crab and among crabs. Mean female cyprid length varied from 366.2 μm (brood 3a) to 420.6 μm (brood 2b), while mean male cyprid length varied from 409.4 μm (brood 2a) to 438.2 μm (brood 2b).

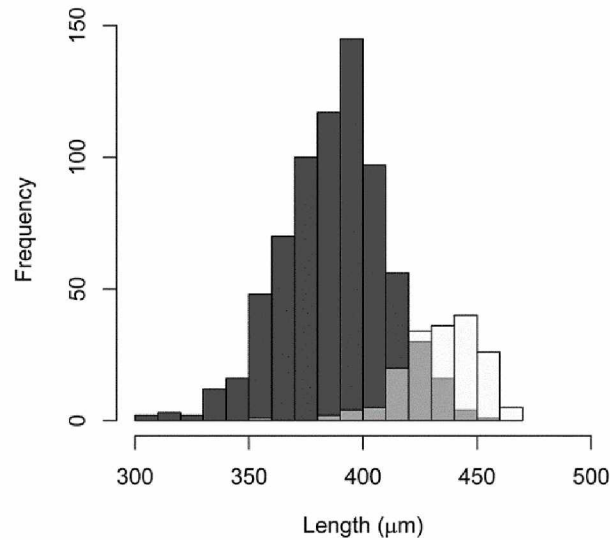


Figure 2.7. Frequency distribution of the length of male (white) and female (dark gray) *Briarosaccus regalis* cyprids, showing the overlap (light gray) in size distribution between sexes.

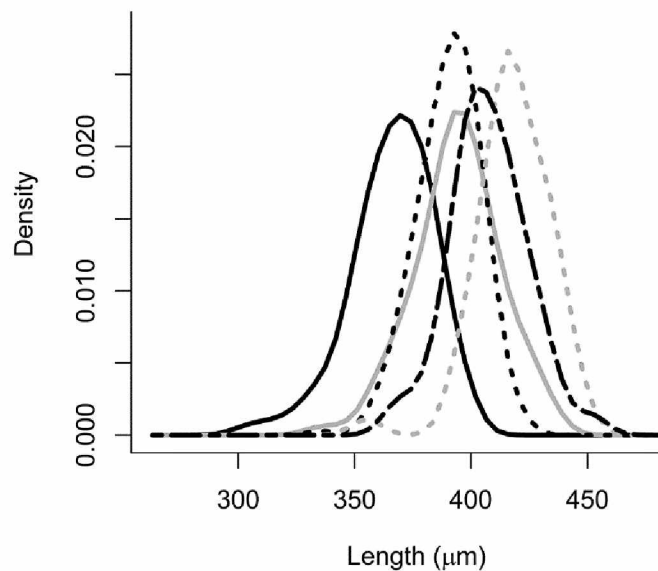


Figure 2.6. Kernel density plots of female *Briarosaccus regalis* cyprids from 5 broods of larvae. Lines indicate individual broods as follows: brood 1e (black, — — —), brood 2a (gray, —), brood 2b (gray, — — —), brood 3a (black, —), and brood 3b (black, — — —). Numbers indicate different host crabs/externa, while letters indicate different broods from the same host crab/externa.

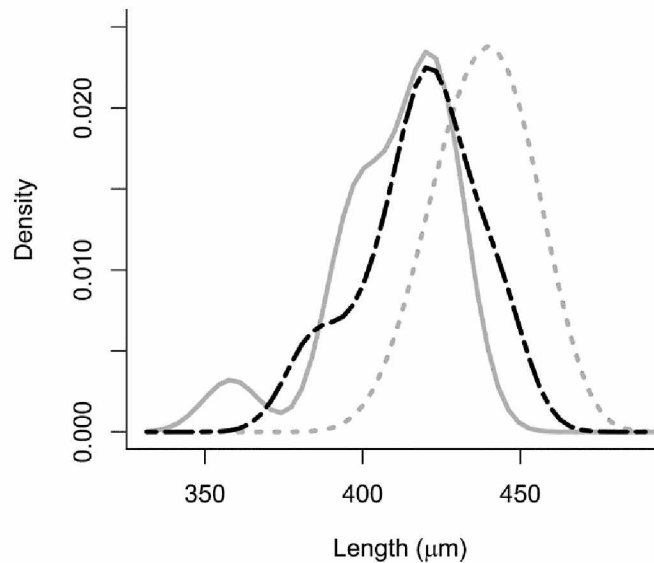


Figure 2.8. Kernel density plots of male *Briarosaccus regalis* cyprids from 3 broods of larvae. Lines indicate individual broods as follows: brood 1e (black, — — —), brood 2a (gray, —), and brood 2b (gray, — — —). Numbers indicate different host crabs/externa, while letters indicate different broods from the same host crab/externa.

Discussion

We examined how two environmental factors, temperature and salinity, could influence the survival and development of *B. regalis*, a parasitic castrator of *P. camtschaticus* and *P. platypus*. The naupliar larvae of *B. regalis* had maximum survival at a wide range of temperatures (4–12 C) and salinities (25–34); in addition, larval development time increased from 11 to 43 days with decreasing temperature. Because the timing of larval sex ratios can be important for fertilization success in rhizocephalans (Ritchie & Høeg, 1981; Poon *et al.*, 2005), we explored methods of sexing cyprid larvae and then examined factors that could influence sex ratios, such as host crab and larval rearing temperature. On average, male cyprids were larger than females, but sizes overlapped considerably such that size was an unreliable predictor of cyprid sex.

For rhizocephalans, the habitat conditions of the hosts generally coincide with the environmental tolerances of the larvae (Kashenko & Korn, 2003). For example, three rhizocephalan species living in Vostok Bay in the Sea of Japan had different temperature tolerance ranges depending on their depth distribution (Kashenko & Korn, 2002a; b, 2003; Kashenko *et al.*, 2002). *Polyascus polygenea*, which lives primarily on intertidal hosts, developed successfully at 18–25 C, while *Peltogaster reticulatus*, which lives from the intertidal to about 10 m, developed at 16–25 C, and *Peltogasterella gracilis*, living from 5 m to several hundred meters, developed at 12–22 C (Kashenko & Korn, 2002a; b, 2003; Kashenko *et al.*, 2002). However, some species of Rhizocephala can tolerate a wider range in environmental variables than would be predicted by their host's habitat. For instance, the host crab *Charybdis callianassa* lives in marine conditions, but its parasite *Heterosaccus lunatus* can develop at salinities of 24–40 (Walker & Lester, 1998). *Briarosaccus regalis* larvae had high survival across fairly wide temperature and salinity ranges, given that host crabs live in deep waters with fairly low variability. However, it is unknown whether *B. regalis* larvae in situ remain at depth where they are released, or migrate into surface waters that can be warmer and fresher. The former appears more probable, given that they are lecithotrophic, and like other members of the Peltogastridae, lack a naupliar eye that is used for phototaxis in other rhizocephalan families (Kashenko & Korn, 2003).

Currently, bottom water temperatures around Alaska are generally on the colder end of what *B. regalis* larvae tolerated in this study (Stone *et al.*, 1992, 1993; Stabenon *et al.*, 2007). King crabs from southeast Alaska, where infected crabs were collected for our experiments, have been recorded living at temperatures between 3.2 and 7.7 C (Stone *et al.*, 1992, 1993). In the Bering Sea (Fig. 2.1) where the largest Alaskan king crab population occurs, bottom water

temperatures on the continental shelf generally range from ≤ -1 to 5 C (Stabeno *et al.*, 2012), but king crabs avoid temperatures ≤ 2 C (Chilton *et al.*, 2010). Based on our results (maximum survival at 4 – 12 C), these temperatures would promote high *B. regalis* larval survival, albeit with relatively slow development.

Briarosaccus regalis larvae had high survival at salinities that they would not typically be exposed to, especially at the higher end of the salinity range (e.g., 37 and 40). In open water on the eastern Bering Sea shelf, salinity variations throughout the water column are minimal, approximately 30–32.5 (Ladd & Stabeno, 2012). Hosts in southeast Alaska occur at a similar salinity range (29.2 to 32.5) (Stone *et al.*, 1992); however, within the enclosed bays and fjords of this region, salinity can be highly variable with season, depth, and location (Carlson, 1980; Etherington *et al.*, 2007; Weingartner *et al.*, 2009). Surface salinities (upper 10 m) can be low (≤ 22) (NOAA: Auke Bay Monitor Station). Since we found *B. regalis* survival to be dramatically reduced at and below a salinity of 22, these surface waters would reduce *B. regalis* survival, and could favor a demersal existence.

The current temperatures and salinities in Alaska waters appear to support high *B. regalis* larval survival; however, our experiments suggest these temperatures may prolong development time. Nauplius survival was not significantly different between 4 and 12 C, but development was 21 days slower at the colder temperature. This extended period of larval development could result in lower larval survival than we detected in our experiments. In the natural environment, planktonic invertebrate larvae are highly vulnerable to mortality from predation, disease, and/or transport into unfavorable habitats (Rumrill, 1990; Vaughn & Allen, 2010). Mortality is generally assumed to increase the longer larvae spend in the plankton (Rumrill, 1990). Thus, faster development rates associated with warmer waters up to 12 C are likely to support higher *B.*

regalis nauplius survival in the wild, although a similar shortening of the cyprid stage in warmer water could reduce the time window for infection.

In rhizocephalans, the sex ratio of successive broods can change seasonally, presumably to synchronize the presence of new (virgin) externa and male larvae, (Ritchie & Høeg, 1981; Poon *et al.*, 2005). For example, *Sacculina sinensis* primarily releases males in the summer and females in the winter (Poon *et al.*, 2005), while *Lernaeodiscus porcellanae* does the opposite (Ritchie & Høeg, 1981). We observed considerable variation in the sex ratio of *B. regalis* broods, but found no indication of seasonality; all-female broods and predominately-male broods were released within days of each other. Photoperiod may trigger sex ratios in other rhizocephalans (Walker & Lester, 2000), but the lithodid hosts of *B. regalis* generally live at depths with low light penetration (Stone *et al.*, 1992), possibly explaining the apparent lack of seasonality. Moreover, *B. regalis* may not need to rely on seasonal cues to synchronize appearance of male larvae and virgin externae, because the virgin externae in this species appear to have a long lifespan. In some species (e.g., *Sacculina carcini*), virgin externae die quickly if they do not acquire a male, while in other species (e.g., *L. porcellanae*), they remain healthy indefinitely (Ritchie & Høeg, 1981). A *P. platypus* with a virgin externa of *B. regalis* was kept in a laboratory for 5 mo (Hawkes *et al.*, 1985), which suggests that virgin externae have considerable time to acquire a male. A mismatch between the presence of virgin externae and male larvae in *B. regalis* is thus unlikely due to the aseasonal variation in sex ratios and the lengthy lifespan of the virgin externae. Furthermore, we found no difference in cyprid sex ratios between larval temperature treatments. While temperature could not have affected sex ratios in our experiments directly because sex is determined in the unfertilized eggs (Høeg, 1995), these

data indicate that larval rearing temperature does not cause differential mortality between the sexes.

There was a large variation in the size of male and female cyprids, both between successive broods from the same host and among hosts. However, this variation did not appear to be seasonal, although our sample size was small and broods were only examined from December to May. Other studies with rhizocephalans have found seasonal differences in cyprid size, with larger larvae occurring in the spring and summer than in the fall (Yanagimachi, 1961). For free-living barnacles, temperature and food availability during the larval stage are both important determinants of cyprid size (Barnes & Barnes, 1965; Anil *et al.*, 2001); however, the lecithotrophic larvae of rhizocephalans, and the lack of size variation between temperature treatments, indicate that these factors were not affecting cyprid size in *B. regalis*. Others have proposed that the temperature during embryo development can affect larval size (Barnes, 1953), yet this also appears unlikely here. For example, broods of female larvae with the largest and smallest mean cyprid size (Fig. 2.7) were exposed to similar and less extreme temperatures during embryo development, compared to other broods (Table 2.1).

Briarosaccus regalis appears well adapted to conditions in the North Pacific where its king crab hosts reside. Continuous aseasonal reproduction and variable sex ratios ensure that male larvae are in the water column when virgin externae emerge, which may take considerable time due to the large host size and cold water temperatures. The large range of temperatures and salinities that these larvae can tolerate give them the ability to survive and infect crabs across a wide latitudinal gradient, from British Columbia (Sloan, 1985) to Norton Sound (C. Lean, pers. comm.), and in diverse habitats with different salinity regimes, ranging from open ocean to fjords and bays. *Briarosaccus regalis* appears well adapted to deal with future increases in

temperature (Royer & Grosch, 2006; Wang *et al.*, 2012) and changes in freshwater runoff and salinity (Jansson *et al.*, 2003; Royer & Grosch, 2006) because it tolerates temperatures and salinities beyond the range that it currently experiences.

In the subarctic Bering Sea, where the largest Alaskan king crab populations occur, sea surface temperature is predicted to increase by approximately 3 C by 2100 (Wang *et al.*, 2012). If this change occurs throughout the water column, *B. regalis* development will accelerate, reducing the length of the planktonic period. *B. regalis* currently infects *P. camtschaticus* and *P. platypus* populations in this region, but prevalence is extremely low (< 0.1% in both species; unpubl. ADF&G observer program data). However, if a shorter development time leads to increased larval survival and infection rates, there could be potential impacts on crab populations and fisheries in the Bering Sea. In order to further understand how *B. regalis* prevalence will change under future climate scenarios, additional studies should focus on other aspects of the life cycle, particularly the process of infection. We also lack an understanding of interacting effects of temperature and salinity, as well as other potential environmental stressors, on both the host and the parasite that would allow us to fully grasp how changing conditions will impact this host-parasite relationship. The present study helps elucidate some environmental tolerances and the overall larval biology of *B. regalis*.

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Literature Cited

- Abello, P., and E. Macpherson. 1992. Epibiosis and rhizocephalan infestation patterns in relation to the reproductive biology of *Lithodes ferox* (Filhol, 1885) (Anomura: Lithodidae). *Journal of Crustacean Biology* **12**: 561–570.
- Anil, A. C., D. Desai, and L. Khandeparker. 2001. Larval development and metamorphosis in *Balanus amphitrite* Darwin (Cirripedia; Thoracica): Significance of food concentration, temperature and nucleic acids. *Journal of Experimental Marine Biology and Ecology* **263**: 125–141.
- Barnes, H. 1953. Size variations in the cyprids of some common barnacles. *Journal of the Marine Biological Association of the United Kingdom* **32**: 297–304.
- Barnes, H., and M. Barnes. 1965. Egg size, nauplius size, and their variation with local, geographical, and specific factors in some common cirripedes. *Journal of Animal Ecology* **34**: 391–402.
- Basson, M. 1994. A preliminary investigation of the possible effects of rhizocephalan parasitism on the management of the crab fishery around South Georgia. *CCAMLR Science* **1**: 175–192.

- Blanford, S., and M. B. Thomas. 2000. Thermal behavior of two acridid species: effects of habitat and season on body temperature and the potential impact on biocontrol with pathogens. *Environmental Entomology* **29**: 1060–1069.
- Bolker, B. M., M. E. Brooks, C. J. Clark, S. W. Geange, J. R. Poulsen, M. H. H. Stevens, and J. S. S. White. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology and Evolution* **24**: 127–35.
- Boudreau, S. A., and B. Worm. 2012. Ecological role of large benthic decapods in marine ecosystems: A review. *Marine Ecology Progress Series* **469**: 195–213.
- Bresciani, J., and J. T. Høeg. 2001. Comparative ultrastructure of the root system in rhizocephalan barnacles (Crustacea: Cirripedia: Rhizocephala). *Journal of Morphology* **249**: 9–42.
- Carlson, H. R. 1980. Seasonal distribution and environment of Pacific herring near Auke Bay, Lynn Canal, Southeastern Alaska. *Transactions of the American Fisheries Society* **109**: 71–78.
- Chilton, E. A., R. J. Foy, and C. E. Armistead. 2010. Temperature effects on assessment of red king crab in Bristol Bay, Alaska, p. 249–263. *In* G.H. Kruse, G.L. Eckert, R.J. Foy, R.N. Lipcius, B. Sainte-Marie, D.L. Stram, and D. Woodby [eds.], *Biology and Management of Exploited Crab Populations Under Climate Change*. Alaska Sea Grant College Program (AK-SG-10-01), Anchorage.
- Etherington, L. L., P. N. Hooge, E. R. Hooge, and D. F. Hill. 2007. Oceanography of Glacier Bay, Alaska: Implications for biological patterns in a glacial fjord estuary. *Estuaries and Coasts* **30**: 927–944.

- Fredensborg, B. L., K. N. Mouritsen, and R. Poulin. 2005. Impact of trematodes on host survival and population density in the intertidal gastropod *Zeacumantus subcarcinatus*. *Marine Ecology Progress Series* **290**: 109–117.
- Glenner, H., J. T. Høeg, A. Klysner, and B. B. Larsen. 1989. Cypris ultrastructure, metamorphosis and sex in seven families of parasitic barnacles (Crustacea: Cirripedia: Rhizocephala). *Acta Zoologica* **70**: 229–242.
- Guernier, V., M. E. Hochberg, and J. F. O. Guegan. 2004. Ecology drives the worldwide distribution of human diseases. *PLOS Biology* **2**: 740–746.
- Guzman, G. L., R. A. Moreno, and H. I. Moyano. 2002. Nuevos hospedadores para *Briarosaccus callosus* Boschma, 1930. *Boletin de la Sociedad de Biologia de Concepcion, Chile* **73**: 83–86.
- Harvell, C. D., C. E. Mitchell, J. R. Ward, S. Altizer, A. P. Dobson, R. S. Ostfeld, and M. D. Samuel. 2002. Climate warming and disease risks for terrestrial and marine biota. *Science* **296**: 2158–2162.
- Hawkes, C. R., T. R. Meyers, and T. C. Shirley. 1985. Larval biology of *Briarosaccus callosus* Boschma (Cirripedia, Rhizocephala). *Proceedings of the Biological Society of Washington* **98**: 935–944.
- Hawkes, C. R., T. R. Meyers, and T. C. Shirley. 1986. Prevalence of the parasitic barnacle *Briarosaccus callosus* on king crabs of southeastern Alaska. *Transactions of the American Fisheries Society* **115**: 252–257.
- Høeg, J. T. 1987. Male cypris metamorphosis and a new male larval form, the trichogon, in the parasitic barnacle *Sacculina carcini* (Crustacea: Cirripedia: Rhizocephala). *Philosophical Transactions of the Royal Society of London* **317**: 47–63.

- Høeg, J. T. 1995. The biology and the life-cycle of the rhizocephala (Cirripedia). *Journal of the Marine Biological Association of the United Kingdom* **75**: 517–550.
- Høeg, J. T., and J. Lützen. 1995. Life cycle and reproduction in the Cirripedia Rhizocephala. *Oceanography and Marine Biology: An Annual Review* **33**: 427–485.
- Isaeva, V., S. Dolganov, and A. Shukalyuk. 2005. Rhizocephalan barnacles—parasites of commercially important crabs and other decapods. *Russian Journal of Marine Biology* **31**: 215–220.
- Jansson, P., R. Hock, and T. Schneider. 2003. The concept of glacier storage: a review. *Journal of Hydrology* **282**: 116–129.
- Kashenko, S. D., and O. M. Korn. 2002a. Effects of temperature and salinity on the larvae of two species of rhizocephalan (Crustacea: Cirripedia). *Invertebrate Reproduction and Development* **41**: 179–184.
- Kashenko, S. D., and O. M. Korn. 2002b. Adaptive responses of the larvae of cirripede barnacle *Peltogasterella gracilis* to changes in seawater temperature and salinity. *Russian Journal of Marine Biology* **28**: 317–323.
- Kashenko, S. D., and O. M. Korn. 2003. Combined effects of seawater temperature and salinity on development of the larvae of the rhizocephalan *Peltogaster reticulatus* (Crustacea: Cirripedia). *Russian Journal of Marine Biology* **29**: 150–155.
- Kashenko, S. D., O. M. Korn, and A. V. Rybakov. 2002. Effects of temperature and salinity on the larvae of *Sacculina polygenea* (Crustacea: Cirripedia: Rhizocephala). *Crustacean Research* **31**: 9–17.
- Kruse, G. H., J. Zheng, and D. L. Stram. 2010. Recovery of the Bristol Bay stock of red king crabs under a rebuilding plan. *ICES Journal of Marine Science* **67**: 1866–1874.

- Kuris, A. M., and K. D. Lafferty. 1992. Modelling crustacean fisheries: Effects of parasites on management strategies. *Canadian Journal of Fisheries and Aquatic Sciences* **49**: 327–336.
- Ladd, C., and P. J. Staben. 2012. Stratification on the Eastern Bering Sea shelf revisited. *Deep Sea Research Part II: Topical Studies in Oceanography* **65**: 72–83.
- Lafferty, K. D. 1993. Effects of parasitic castration on growth, reproduction and population dynamics of the marine snail *Cerithidea californica*. *Marine Ecology Progress Series* **96**: 229–237.
- Lázaro-Chávez, E., F. Alvarez, and C. Rosas. 1996. Records of *Loxothylacus texanus* (Cirripedia: Rhizocephala) parasitizing the blue crab *Callinectes sapidus* in Tamiahua Lagoon, Mexico. *Journal of Crustacean Biology* **16**: 105–110.
- Lazzaro, B. P., H. A. Flores, J. Lorigan, and C. Yourth. 2008. Genotype-by-environment interactions and adaptation to local temperature affect immunity and fecundity in *Drosophila melanogaster*. *PLOS Pathology* **4**: e1000025.
- Morado, J. F. 2011. Protistan diseases of commercially important crabs: A review. *Journal of Invertebrate Pathology* **106**: 27–53.
- Morado, J. F., C. A. Shavey, R. Tatyana, and V. C. Lowe. 2014. Chapter 6. Diseases of king crabs and other anomalies, p. 139–210. *In* B.G. Stevens [ed.], *King Crabs of the World: Biology and Fisheries Management*. CRC Press, Boca Raton.
- Moyse, J., J. T. Høeg, P. G. Jensen, and H. A. H. Al-Yahya. 1995. Attachment organs of cypris larvae: Using scanning electron microscopy. *Crustacean Issues* **10**: 153–177.
- Murdock, C. C., K. P. Paaijmans, A. S. Bell, J. G. King, J. F. Hillyer, A. F. Read, and M. B. Thomas. 2012. Complex effects of temperature on mosquito immune function. *Proceedings of the Royal Society B: Biological Sciences* **279**: 3357–3366.

- Negovetich, N. J., and G. W. Esch. 2008. Quantitative estimation of the cost of parasitic castration in a *Helisoma anceps* population using a matrix population model. *The Journal of Parasitology* **94**: 1022–30.
- Noever, C., A. Olson, and H. Glenner. 2016. Two new cryptic and sympatric species of the king crab parasite *Briarosaccus* (Cirripedia: Rhizocephala) in the North Pacific. *Zoological Journal of the Linnean Society* **176**: 3–14.
- Oug, E., S. K. J. Cochrane, J. H. Sundet, K. Norling, and H. C. Nilsson. 2011. Effects of the invasive red king crab (*Paralithodes camtschaticus*) on soft-bottom fauna in Varangerfjorden, northern Norway. *Marine Biodiversity* **41**: 467–479.
- Pearce, J. and N. Balcom. 2005. The 1999 Long Island Sound lobster mortality event: findings of the comprehensive research initiative. *American Malacological Bulletin* **24**: 361–397.
- Poon, D. Y. N., G. Walker, and B. K. K. Chan. 2005. Distribution, adult morphology, and larval development of *Sacculina sinensis* (Cirripedia: Rhizocephala: Kentrogonida) in Hong Kong coastal waters. *Journal of Crustacean Biology* **25**: 1–10.
- R Core Team. 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Australia.
- Reisser, C. E., and R. B. Forward. 1991. Effect of salinity on osmoregulation and survival of a rhizocephalan parasite, *Loxothylacus panopaei*, and its crab host, *Rhithropanopeus harrisi*. *Estuaries* **14**: 102–106.
- Ritchie, L., and J. T. Høeg. 1981. The life history of *Lernaodiscus porcellanae* (Cirripedia: Rhizocephala) and co-evolution with its porcellanid hosts. *Journal of Crustacean Biology* **1**: 334–347.

- Royer, T. C., and C. E. Grosch. 2006. Ocean warming and freshening in the northern Gulf of Alaska. *Geophysical Research Letters* **33**: 1–6.
- Rumrill, S. S. 1990. Natural mortality of marine invertebrate larvae. *Ophelia* **32**: 163–198.
- Schneider, C. A., W. S. Rasband, and K. W. Eliceiri. 2012. NIH image to imageJ: 25 years of image analysis. *Nature Methods* **9**: 671–675.
- Sheehan, K. L., K. D. Lafferty, J. O'Brien, and J. Cebrian. 2011. Parasite distribution, prevalence, and assemblages of the grass shrimp, *Palaemonetes pugio*, in southwestern Alabama, USA. *Comparative Parasitology* **78**: 245–256.
- Sloan, L. M., S. V. Anderson, and B. Pernet. 2010. Kilometer-scale spatial variation in prevalence of the rhizocephalan *Lernaeodiscus porcellanae* on the porcelain crab *Petrolisthes cabrilloi*. *Journal of Crustacean Biology* **30**: 159–166.
- Sloan, N. A. 1984. Incidence and effects of parasitism by the rhizocephalan barnacle, *Briarosaccus callosus* Boschma, in the golden king crab, *Lithodes aequispina* Benedict, from deep fjords in northern British Columbia, Canada. *Journal of Experimental Marine Biology and Ecology* **84**: 111–131.
- Sloan, N. A. 1985. Life history characteristics of fjord-dwelling golden king crabs *Lithodes aequispina*. *Marine Ecology Progress Series* **22**: 219–228.
- Sparks, A. K., and J. F. Morado. 1986. Histopathology and host response in lithodid crabs parasitized by *Briarosaccus callosus*. *Diseases of Aquatic Organisms* **2**: 31–38.
- Stabeno, P. J., N. A. Bond, and S. A. Salo. 2007. On the recent warming of the southeastern Bering Sea shelf. *Deep Sea Research Part II: Topical Studies in Oceanography* **54**: 2599–2618.

- Stabeno, P. J., N. B. Kachel, S. E. Moore, J. M. Napp, M. Sigler, A. Yamaguchi, and A. N. Zerbini. 2012. Comparison of warm and cold years on the southeastern Bering Sea shelf and some implications for the ecosystem. *Deep Sea Research Part II: Topical Studies in Oceanography* **65–70**: 31–45.
- Stevens, B.G. and Lovrich, G.A. 2014. Chapter 1: King crabs of the world: species and distributions. In: *King Crabs of the World, Biology and Fisheries Management* (B. G. Stevens, ed), pp. 1–30. CRC Press, Boca Raton.
- Stone, R. P., C. E. Oclair, and T. C. Shirley. 1992. Seasonal migration and distribution of female red king crabs in a Southeast Alaskan estuary. *Journal of Crustacean Biology* **12**: 546–560.
- Stone, R. P., C. E. Oclair, and T. C. Shirley. 1993. Aggregating behavior of ovigerous female red king crab, *Paralithodes camtschaticus*, in Auke Bay, Alaska. *Canadian Journal of Fisheries and Aquatic Sciences* **50**: 750–758.
- Thomas, M. B., and S. Blanford. 2003. Thermal biology in insect-parasite interactions. *Trends in Ecology and Evolution* **18**: 344–350.
- Tindle, S., E. Boone, J. O'Brien, and A. Boettcher. 2004. Effects of salinity on larval stages of the rhizocephalan barnacle *Loxothylacus texanus*: Survival and metamorphosis in response to the host, *Callinectes sapidus*. *Journal of Experimental Marine Biology and Ecology* **302**: 165–176.
- Vaughn, D., and J. D. Allen. 2010. The peril of the plankton. *Integrative and comparative biology* **50**: 552–570.
- Walker, G., and A. S. Clare. 1994. The effect of salinity on the development of *Loxothylacus panopaei* larvae (Crustacea: Cirripedia: Rhizocephala). *Estuaries* **17**: 276–282.

- Walker, G., A. S. Clare, D. Rittschof, and D. Mensching. 1992. Aspects of the life-cycle of *Loxothylacus panopaei* (Gissler), a sacculinid parasite of the mud crab *Rhithropanopeus harrisi* (Gould): A laboratory study. *Journal of Experimental Marine Biology and Ecology* **157**: 181–193.
- Walker, G., and R. J. G. Lester. 1998. Effect of salinity on development of larvae of *Heterosaccus lunatus* (Cirripedia: Rhizocephala). *Journal of Crustacean Biology* **18**: 650–655.
- Walker, G., and R. J. G. Lester. 2000. The cypris larvae of the parasitic barnacle *Heterosaccus lunatus* (Crustacea, Cirripedia, Rhizocephala): Some laboratory observations. *Journal of Experimental Marine Biology and Ecology* **254**: 249–257.
- Wang, M., J. E. Overland, and P. Stabeno. 2012. Future climate of the Bering and Chukchi Seas projected by global climate models. *Deep Sea Research Part II: Topical Studies in Oceanography* **65**: 46–57.
- Weingartner, T., L. Eisner, G. L. Eckert, and S. Danielson. 2009. Southeast Alaska: oceanographic habitats and linkages. *Journal of Biogeography* **36**: 387–400.
- Yanagimachi, R. 1961. Studies on the sexual organization of the Rhizocephala. III. The mode of sex determination in *Peltogasterella*. *Biological Bulletin* **120**: 272–283.

CHAPTER 3: METABOLITE VARIATION IN KING CRAB INFECTED WITH A PARASITIC BARNACLE²

Abstract

Parasitic barnacles, the Rhizocephala, infect and castrate their crustacean hosts, causing changes in host morphology, physiology, and behavior. However, the physiological processes by which rhizocephalans manipulate their hosts are not well understood. We used an untargeted LC-MS metabolomics approach to compare the metabolite profiles (e.g., small molecules such as signaling molecules and other products of metabolism) of lithodid crab with and without rhizocephalan infections. We tested whether metabolite changes caused by rhizocephalan infection could be detected in the hemolymph, muscle, and hepatopancreas of *Paralithodes camtschaticus* (red king crab) and the hemolymph of *Lithodes aequispinus* (golden king crab) that are infected by the rhizocephalans *Briarosaccus regalis* and *B. auratum*, respectively. King crab tissue and hemolymph samples were collected between 2013 and 2015 in Southeast Alaska. In all analyzed tissues, hundreds of putative metabolites were identified, yet few differed with crab sex and no metabolites could differentiate infected and healthy crab, regardless of crab sex. Five metabolites, including a putatively identified histidine tripeptide and an aminobenzoic acid, had an interaction between sex and infection state. There were large variations in the crab metabolome with collection year and location, which likely masked differences between sex and infection status. This environmental metabolomics approach suggests that the crab metabolome may be greatly influenced by environmental variation. Future studies in a more controlled

² Zacher, L.S., L. Horstmann, S.M. Hardy. Metabolite variation in king crab infected with a parasitic barnacle. Prepared for submission to Journal of Crustacean Biology

environment will be necessary to further unravel how the host crab metabolome changes with rhizocephalan infection.

Introduction

The Rhizocephala is a taxon of highly specialized parasitic cirripeds (barnacles) that infect other crustaceans, primarily anomuran and brachyuran crab (Høeg, 1995). The larval stage is similar to other cirripeds, with several free-living naupliar instars, and a cyprid that searches for suitable settlement substrate (Høeg & Møller, 2006). Female cyprids settle on host crab and inject parasitic cells into the host hemolymph, with some species first metamorphosing into another larval stage, the kentrogon (Høeg & Lützen, 1995; Glenner, 2001). The injected cells develop into an internal rootlet system (the interna), which spreads throughout tissues of the host (Noever *et al.*, 2016). Nutrients are absorbed directly from the host (Payen *et al.*, 1983; Shukalyuk & Isaeva, 2000; Bresciani & Høeg, 2001). When mature, an external reproductive structure (the externa) forms, generally under the host's abdomen (Høeg & Lützen, 1995). Rhizocephalans infect both male and female hosts, causing degeneration of the host's gonads and sterilization (Høeg, 1995).

Rhizocephalans impose substantial control over their host's morphology, physiology, and behavior. Although specifics vary among species, host growth is often decreased or suspended, and males are generally feminized, adopting female egg-caring behaviors and becoming morphologically more similar to females (e.g., wider abdominal flaps; (Høeg, 1995)). Infected crab of both sexes tend to the externa, as if it were their own brood, cleaning and aerating the eggs (Ritchie & Høeg, 1981; deVries *et al.*, 1989). Although the morphological and behavioral changes in host crab have been well documented, the underlying pathophysiology and mechanisms of "host control" are poorly understood. Endocrine organs, gonads, and the central

nervous system are generally disturbed during infection, either by direct penetration of rootlets (Sparks & Morado, 1986; Noever *et al.*, 2016) or by the release of a diffusible substance from the rootlets without direct contact (Rubiliani & Godette, 1981; Rubiliani, 1985). Although differences occur among host species and tissue types, protein synthesis, composition, and body concentrations change with rhizocephalan infection (Rubiliani & Godette, 1981; Powell & Rowley, 2008). In addition, for some crab species metabolites like glucose, glycogen, and triacylglycerol differ between healthy and in infected crab (Shirley *et al.*, 1986; Powell & Rowley, 2008; Hsiao *et al.*, 2016). In *Metopograpsus thukuhar* (Owen, 1839) infected with the rhizocephalan *Polyascus plana* (Boschma, 1933), glucose, glycogen, triacylglycerol were higher in the hepatopancreas of parasitized crab; from here nutrients are likely transported into the interna rootlets, which are concentrated in this region (Hsiao *et al.*, 2016).

The goal of this study was to explore the utility of an environmental metabolomics approach (Lankadurai *et al.*, 2013) to obtain a more holistic view of the effects of rhizocephalan infection on the crab metabolome under field conditions. Metabolomics is a newly emerging field, especially outside of human medicine, which allows for the simultaneous measurement of hundreds to thousands of metabolites, low molecular weight molecules (e.g., enzyme substrates/products, hormones, amino acids; Lindon *et al.* 2007). Metabolites are the ultimate product of gene expression (Silva *et al.*, 2017). The metabolome defines an organism's overall physiological status, making this a useful technique to characterize physiological reactions to foreign substances, such as toxins, pharmaceuticals, or disease (Kaddurah-Daouk *et al.*, 2008). This is the first time metabolomics has been applied to the rhizocephalan host-parasite relationship; however, this technique has been successfully applied in other host-parasite systems

(Rosenblum *et al.*, 2005; Kafsack & Llinás, 2010), and in several crustacean species (Schock *et al.*, 2010; Poynton *et al.*, 2011; Hammer *et al.*, 2012; Maity *et al.*, 2012).

Here, we tested whether metabolite signals of a rhizocephalan infection can be detected in host crab hemolymph, muscle, and hepatopancreas. These tissues were chosen, because they exhibit variation in metabolite concentrations with rhizocephalan infection (Shirley *et al.*, 1986; Hsiao *et al.*, 2016), and they have different degrees of interna rootlet penetration (Noever *et al.*, 2016). Most metabolomics studies of environmental stressors (reviewed in Lankadurai *et al.*, 2013), and previous stressor studies with decapods (Schock *et al.*, 2010; Hammer *et al.*, 2012), use tightly controlled laboratory conditions to quantify metabolic effects of the stressor (Schock *et al.*, 2010; Hammer *et al.*, 2012). However, the goal of this study was to examine the metabolite signals of infection in conjunction with natural environmental variations (i.e., using field collected samples).

This study was conducted in two lithodid species, *Paralithodes camtschaticus* (Tilesius, 1815), red king crab, and *Lithodes aequispinus* Benedict, 1895, golden king crab, which are infected by *Briarosaccus regalis* Noever, Olson & Glenner, 2016 and *B. auratum* Noever, Olson & Glenner, 2016, respectively (previously both *B. callosus* Boschma, 1930). These two crab species support valuable commercial fisheries (Orensanz *et al.*, 1998; Stevens, 2014) and are native to subarctic waters in the North Pacific, although *P. camtschaticus* has also been introduced in the Barents's Sea (Oug *et al.*, 2011; Stevens & Lovrich, 2014). Infections of these two king crab species by *Briarosaccus* spp. have been observed throughout the native range of the hosts, with prevalence varying from < 1% to 41% (external manifestation of prevalence; Sloan, 1984, 1985; Hawkes *et al.*, 1986; Isaeva *et al.*, 2005; Shukalyuk *et al.*, 2005). The physiology of this host-parasite relationship is not well understood, despite the commercial

importance of the king crab hosts. Concentrations of hemocyanin, protein, and glucose in the hemolymph increase with *Briarosaccus* infection for both crab species (Shirley *et al.*, 1986). In addition, *Briarosaccus* infection causes the most obvious morphological changes in male crab, which include a smaller size, slightly broader abdomen, growth of coxal setae, and smaller chelipeds (Reinhard, 1956; Sloan, 1984; Hawkes *et al.*, 1986). Behavior may also be altered, such that infected males occur at the same depths as females (Sloan, 1984). Given the commercial importance of these crab species and the potential for high parasite prevalence, this study explores the utility of identifying biomarkers that can reveal parasite prevalence when there is no external sign of infection.

Methods

Sample collection

All king crab were caught with crab pots in southeast Alaska (sample sizes in Table 3.1). Infected and non-infected *P. camtschaticus* were caught June–July of 2013, 2014, and 2015 during the Alaska Department of Fish and Game (ADF&G) red king crab pot survey in the vicinity of Juneau, Alaska (Fig. 3.1; maps created using ArcGIS version 10.3; ESRI, 2014).

Table 3.1. Sample sizes for healthy and infected (by *Briarosaccus* spp.) *Paralithodes camtschaticus* and *Lithodes aequispinus* of both sexes. Total samples sizes for each tissue and hemolymph are given in bold. In parentheses for *P. camtschaticus*, the sample size is broken down into crab collected in 2013, 2014, and 2015 from Juneau, Alaska, followed by the number of crab that were held in the lab for one year before samples were taken in 2015. For *L. aequispinus*, samples were only collected in 2014 and 2015 near Ketchikan, Alaska.

	<i>P. camtschaticus</i>			<i>L. aequispinus</i>
	Hemolymph	Hepatopancreas	Muscle	Hemolymph
Infected Female	12 (2/1/8/1)	11 (2/0/8/1)	11 (2/0/8/1)	9 (5/4)
Healthy Female	11 (2/2/5/2)	8 (2/0/5/1)	9 (2/0/5/2)	6 (2/4)
Infected Male	15 (2/3/8/2)	13 (2/1/8/2)	13 (2/1/8/2)	4 (2/2)
Healthy Male	14 (1/6/5/2)	10 (1/2/5/2)	10 (1/2/5/2)	6 (5/1)

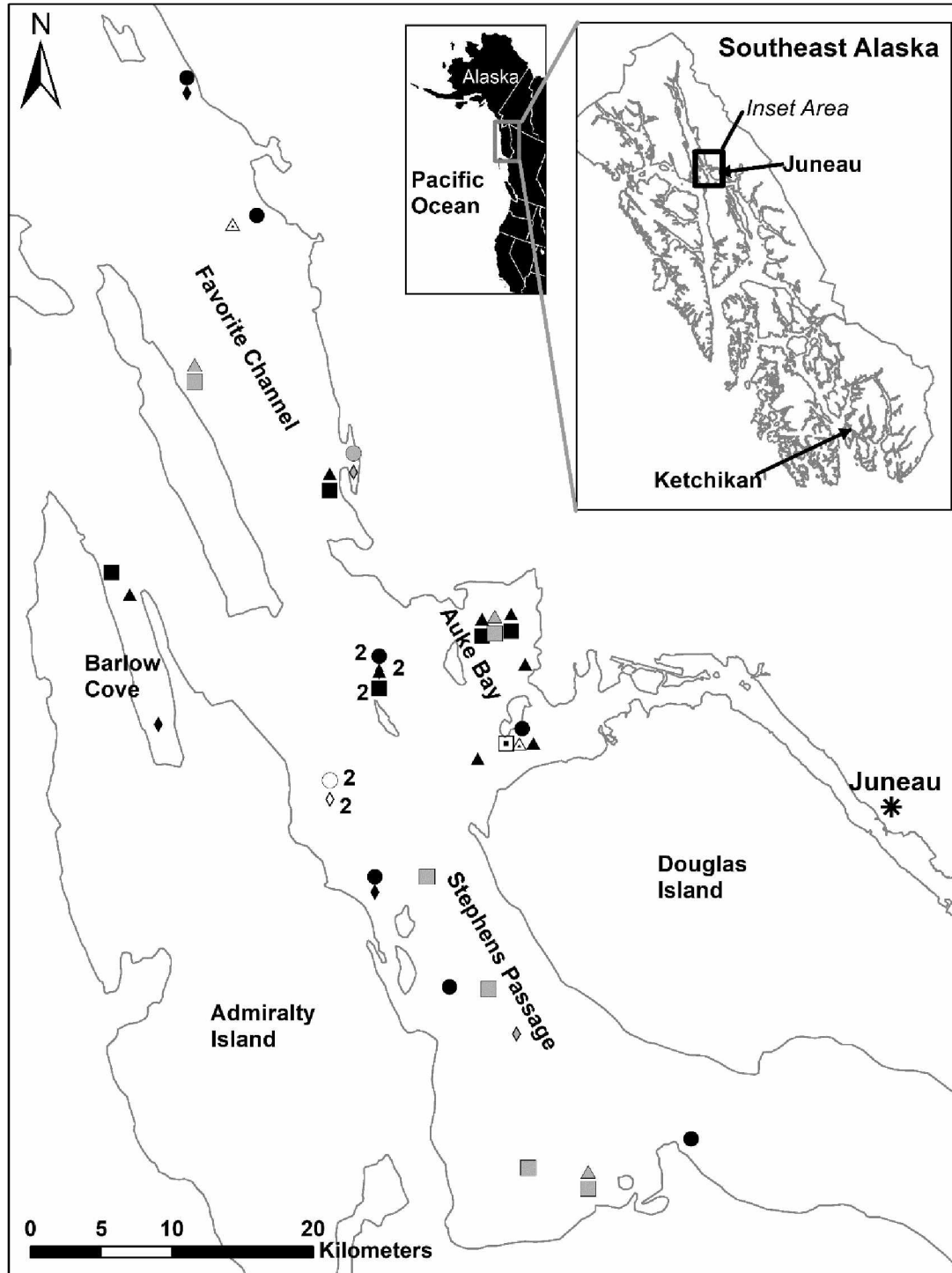


Figure 3.1. Map of southeast Alaska, showing collection locations of *Paralithodes camtschaticus* around Juneau, Alaska, over three years (2013 – 2015). Crab were healthy or infected with the parasite, *Briarosaccus regalis*. Squares = infected males, triangles = healthy males, circles = infected females, diamonds = healthy females; open symbols = 2013, gray symbols = 2014, black symbols = 2015. Numbers occur next to symbols when more than one crab of the same sex and infection status was caught in the same location.

During the 2013, 2014, and 2015 surveys, temperature was 4.9 ± 0.007 °C (mean \pm 1 SE), 5.4 ± 0.008 °C, and 5.9 ± 0.008 °C, respectively. Infected and non-infected *L. aequispinus* were sampled by ADF&G observers during the commercial fishery in March 2014 and 2015 in the southern golden king crab management area near Ketchikan, Alaska. Mean prevalence of crab with externae and scars from lost externae in these areas during sample collection years was 0.31% for *P. camtschaticus* and 3.01% (2014 only) for *L. aequispinus* (unpublished ADF&G survey and observer data). All crab had hard shells and carapace length measurements identified them as adults (*L. aequispinus*: females = 130.8 ± 21.2 mm, males = 150.3 ± 24.8 mm [mean carapace length \pm 1 SD]; *P. camtschaticus*: females = 129.2 ± 8.0 mm, males = 129.8 ± 21.8). Tissue samples were collected as soon as crab were brought on board a vessel; however, in 2014, seven *P. camtschaticus* (3 infected, 4 healthy) were brought back to the Seward Marine Center (Seward, Alaska) and kept in flow through tanks under ambient seawater conditions for one year (Sloan & Hardy, 2017). For these crab, tissue samples were taken a few days prior to the 2015 collection trip for *P. camtschaticus*. When feasible, crab were collected and tissues were sampled in pairs, one infected and one non-infected crab of the same sex, similar size, and similar collection location. For *P. camtschaticus*, samples were taken from the hepatopancreas, muscle, and hemolymph, while only hemolymph samples were taken from *L. aequispinus* (due to ADF&G observer time limitations). Infected crab were identified by the presence of an externa or a scar from an externa that had fallen off. *P. camtschaticus* without an externa or scar were dissected to verify the absence of an interna before being considered non-infected. Because only hemolymph was extracted from *L. aequispinus*, they were not dissected and crab were considered non-infected, if they lacked external signs of infection. Given the low prevalence of crab with externae and scars during the collection years, it is unlikely that any crab with early

internal stages of infection were misidentified as healthy crab; nevertheless, this possibility was taken into consideration during data analyses.

Within 30 minutes of pot recovery, tissue and hemolymph samples were collected using sterile, disposable sampling supplies and placed in sterile 2 mL cryogenic vials. Hepatopancreas samples (~20 g) were collected from the dorsal surface of crab after removing the carapace and membranous tissues. The interna of *Briarosaccus* is interwoven within the tubules of the hepatopancreas. Separating these in a timely manner during field collection was not possible, so hepatopancreas samples from infected crab likely include *Briarosaccus* tissue as well as crab tissue. Muscle samples (~20 g) were collected from the merus segment of a leg. The interna of *Briarosaccus* does not extend into the legs (Noever *et al.*, 2016), thus muscle samples only included crab tissue. Hemolymph samples (~2 mL) were taken by inserting a sterile 22 gauge hypodermic syringe into a leg joint. Upon collection, all samples were immediately flash frozen in a liquid nitrogen dry shipper (< -80 °C) and later stored at -80 °C at the University of Alaska Fairbanks until tissue preparation and analysis.

Sample extraction

We extracted duplicate (analytical replicates) 100 mg sub-samples from all field samples. Sub-samples were first removed with sterilized stainless steel instruments and weighed. Then, samples were homogenized with 200 µL double distilled water in homogenizer vials with ceramic beads (1.5 mm) at 6.5 m/s over 5 1-min intervals (chilled on ice for 2-3 mins between each repeat). Next, 300 µL methanol and 500 µL chloroform were added to the vials and vortexed for 3 min. Vials were then centrifuged at 8,000 RPM for 15 min at 4 °C to separate polar (upper) and non-polar (lower) fractions, which were then pipetted into separate cryovials

and evaporated under nitrogen. Dried samples were stored at -80 °C until metabolomics analysis.

Metabolomic analyses

Reverse phase liquid chromatography mass spectrometry (LC-MS) with positive electrospray ionization (ESI) was used for metabolite analysis. Analyses were performed at the Metabolite Profiling Facility in the Bindley Bioscience Center at Purdue University. An Agilent 6545 Quadrupole Time of Flight (Q-ToF) mass spectrometer (Agilent Technologies, Santa Clara, CA) was coupled to an Agilent 1290 Infinity II UPLC equipped with a thermostatted well plate auto sampler and binary pumping device. All methods utilized Agilent MassHunter software (version B.06.01). The jet stream electrospray source conditions were as follows: gas temperature 320 °C, gas flow rate 8 L/min, nebulizer pressure 35 psi, sheath gas temperature 320 °C, sheath gas flow rate 8 L/min, capillary voltage 3500 V, fragmentor voltage 135 V, and skimmer voltage 65 V. A reference mass solution was infused by dual ESI spray needle. Reference masses 121.050873 and 922.009798 were used during the analysis.

For polar samples, an Acquity UPLC HSS T3 column (Waters Corp., Milford, MA) with 2.1 x 100 mm, 1.8 µm dimensions was used for the separation. Solvent A consisted of water + 0.1% formic acid. Solvent B consisted of acetonitrile + 0.1% formic acid. Samples were reconstituted with a mixture of solvents A and B (95% A, 5% B). The flow rate was 450 µL/min. A volume of 5 µL was loaded onto the column. The gradient was as follows: time 0 min, 0% B; time 1 min, 0% B; time 20 min, 30% B; time 21 min, 95% B; time 21.5 min, 0% B; time 25 min, 0% B. In MS full scan mode, data were acquired with a mass range of 70-1000 m/z and a scan rate of 1.2 spectra/sec.

For non-polar samples, an Acquity UPLC BEH C18 column (Waters Corp., Milford, MA) with 2.1 x 100 mm, 1.7 μ m dimensions was used for the separation. Solvent A consisted of water + 10 mM ammonium acetate + 0.1% formic acid. Solvent B consisted of acetonitrile/isopropyl alcohol (50/ 50 v/v) + 10 mM ammonium acetate + 0.1% formic acid. Samples were reconstituted with 100 μ L of a 50/50 mixture of solvent A and B. The flow rate was 400 μ L/min. A volume of 8 μ L was loaded onto the column. The gradient was as follows: time 0 min, 35% B; time 0.5 min, 35% B; time 5 min, 80% B; time 10 min, 100% B; time 15 min, 100% B; time 17 min, 35% B; time 20 min, 35% B. In MS full scan mode, data were acquired with a mass range of 100-1200 m/z and a scan rate of 1.2 spectra/sec.

Data analyses and processing

Raw mass spectrometry data consist of mass spectra (relative abundance of each mass-to-charge (m/z) ratio) at specific retention times (Barnes *et al.*, 2016). During extraction of raw data, ions are identified, which have a unique neutral mass, retention time, and abundance. We define molecular features as groups of related, covariant ions, which tentatively represent unique compounds (Sana *et al.*, 2013). Molecular features consist of multiple ions with related m/z ratios, because they correspond to different isotopes, adducts, or aggregates of the same molecule (Sana *et al.*, 2008). Raw data were first processed using MassHunter Qualitative Analysis software (version B.06.00, Agilent Technologies, Santa Clara, CA, USA). Samples from each crab species, tissue type, and polarity (polar/nonpolar) were processed separately. Total ion chromatograms were viewed, and in rare cases (2 of 162 samples) one of the analytical replicates was removed from the sample set in either the polar or non-polar fractions due to large inconsistencies in chromatograms. Molecular features were extracted using the molecular feature extractor (MFE), an untargeted data-mining algorithm, which removes background noise,

identifies co-variant ions, looks for isotopes and specific adducts (H^+ , Na^+ , and [non-polar only] NH_4^+), and groups them together into molecular features with a unique neutral mass and retention time. Minimum peak height was set at 600 counts for the polar phase and 1,000 counts for non-polar phase after examining background noise level. Charge state was set at 1, the minimum abundance was an absolute height of 5,000 counts, the lowest number of ions allowed in the isotopic distribution was set at 2, and the peak spacing tolerance was $0.0025\ m/z \pm 14\ ppm$ (isotope model “common organic molecules”). For each sample, data files with the extracted features were exported as CEF files and imported into Mass Profiler Professional (MPP; version B.07.00), Agilent Technologies, Santa Clara, CA, USA. In MPP, samples were grouped into four conditions (healthy male, healthy female, infected male, infected female). Molecular features were aligned across all samples using a retention time window of 0.25 min and a mass tolerance window of $15\ ppm \pm 2\ mDa$. Aligned features were then filtered to include only features that occurred in at least 50% of the samples in at least one condition. This feature list was used in MassHunter Qualitative Analysis for recursive analysis, using the “find by ion” algorithm. This targeted data mining approach can help reduce the number of false negative results. The original data were re-mined using this algorithm, with a mass window of 15 ppm and a retention time 0.25 min. Then, resulting CEF files were reimported into MPP and then grouped and aligned as before. During alignment all features were checked manually to ensure proper alignment. To reduce the number of zero entries and eliminate features that could have been caused by noise, features were filtered using a modified 80% rule, where the feature was kept if it had a nonzero value for at least 80% of the samples in at least one of the four conditions (Yang *et al.*, 2015). At this point, analytical replicates were averaged to create a single record of features for each sample. Raw data, including m/z , retention time, and relative abundance were

exported into CSV files and polar and nonpolar data from each species and tissue type were combined for statistical analysis.

Statistical analysis and feature identification

Statistical analyses were performed in Metaboanalyst 3.5 (Xia *et al.*, 2015). Peak intensity tables were uploaded and processed prior to statistical analyses. Missing data were replaced with one half the minimum detected value (Xia *et al.*, 2009). Data were filtered by removing features with near-constant values using the interquartile range (10% filtered if 250 – 500 features; 25% filtered if >500 features), and data were scaled using parento scaling (mean centered and divided by the square root of the standard deviation of each feature; Yang *et al.* 2015). Principle components analysis (PCA) was used as an unsupervised multivariate analysis technique (groups were not pre-defined), while the partial least squares discriminate analysis (PLS-DA) was performed as a supervised method to identify features that specifically discriminated among the four conditions. PLS-DA models were validated using a permutation test (2,000 permutations), and the number of components in the models were chosen using 10-fold cross validation (R^2 and Q^2). Univariate two-way analysis of variance (ANOVA; $\alpha = 0.05$) with Tukey's post hoc test was used to identify significant features that differed among the four conditions. False discovery rate corrections for multiple comparisons were applied to all reported p-values (Benjamini & Hochberg, 1995). Initial tests were performed to determine if differences could be detected between scarred crab and those with an externa. Because no differences were found ($p < 0.05$), both scarred crab and those with an externa were treated as infected in all analyses.

For features with significant differences among conditions, the “ID Browser” in MPP was used to generate molecular formulae and identify compounds (level 3 identification; Sumner

et al., 2007) using accurate mass (mass tolerance < 10 ppm), isotope ratios, abundance, and spacing. Molecular formulae were generated using an algorithm that combines C (number of atoms allowed: 0-60), H (0-120), N (0-30), O (0-30), S (0-3), and Cl (0-3). Molecular formulae were searched in the Agilent METLIN database, identifying one or more putative metabolites. Only the compounds with the highest score (i.e., most likely to be the true metabolite) were considered as putative identities of features. For features with multiple potential identities with equally high scores, only the most plausible are reported (e.g., synthetic compounds, rare metabolites eliminated).

Results

The metabolomes of *P. camtschaticus* hemolymph, muscle, and hepatopancreas and *L. aequispinus* hemolymph were characterized and compared among four conditions – male and female crab that were either healthy or infected with a *Briarosaccus* spp. After the quality control filters were applied to remove noise, 270, 300, and 444 features remained for *P. camtschaticus* hemolymph, hepatopancreas, and muscle, respectively, and 390 features in *L. aequispinus* hemolymph. Both multivariate and univariate tools were then employed to identify differences among sexes and infection states.

In the PCA, 40 to 66% of the total variance in the data was explained by the first two principle components for all tissue types and for both species (Fig. 3.2). There was no apparent clustering based on sex or infection state. Instead, the obvious clusters (especially in the hemolymph) resulted from collection year and location (Fig. 3.3). For all sample types, supervised PLS-DA score plots showed more clustering, especially between the sexes, yet permutation tests showed that these patterns could have arisen by chance ($p > 0.1$) in all tissues except muscle ($p = 0.018$). For muscle, the two-component model was selected as the optimized

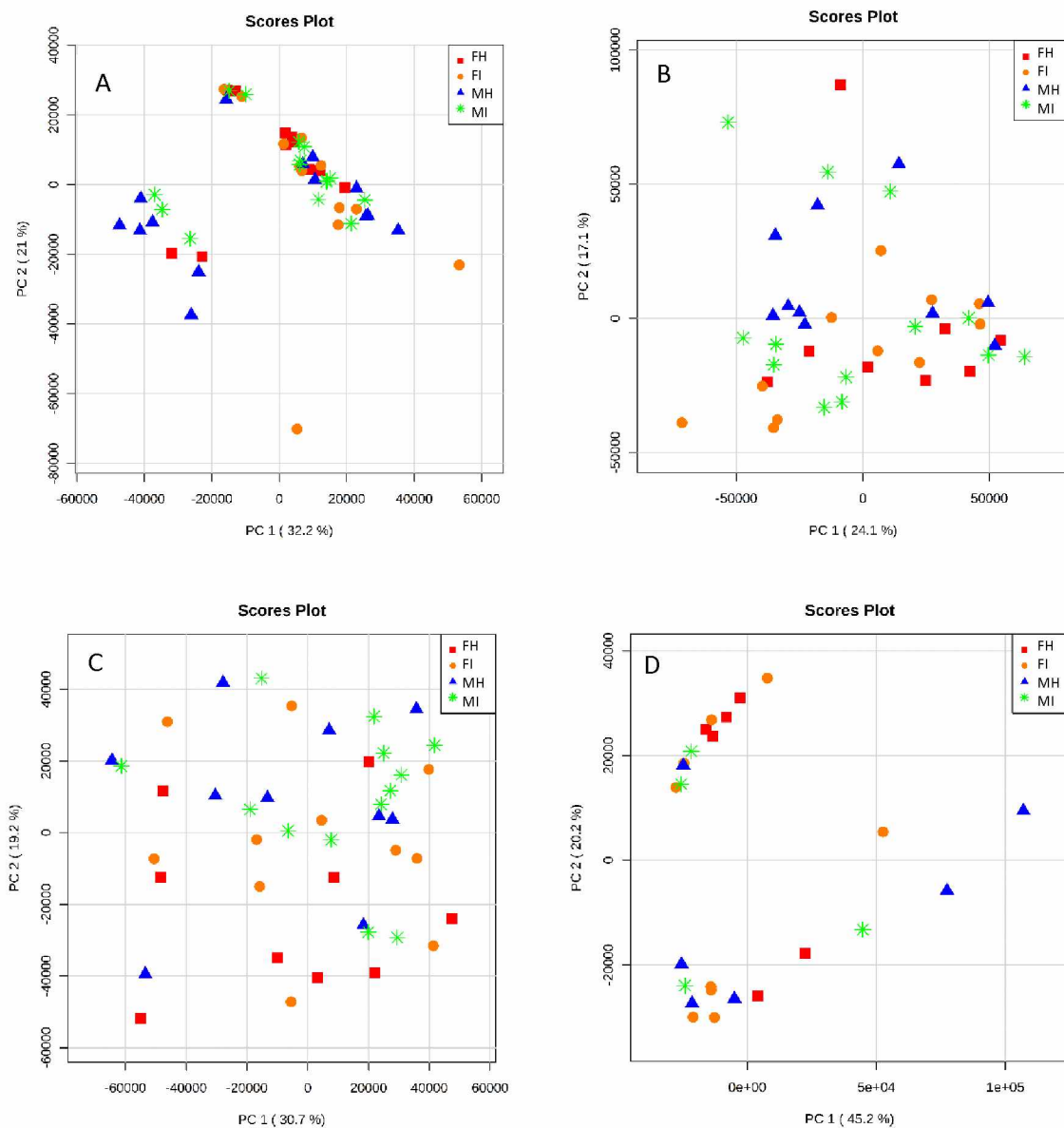


Figure 3.2. Principle components analysis score plots for uniquely identified features (potential metabolites) in A) *P. camtschaticus* hemolymph, B) *P. camtschaticus* hepatopancreas, C) *P. camtschaticus* muscle, and D) *L. aequispinus* hemolymph. Score plot symbols show healthy females (FH), infected females (FI), healthy males (MH), and infected males (MI).

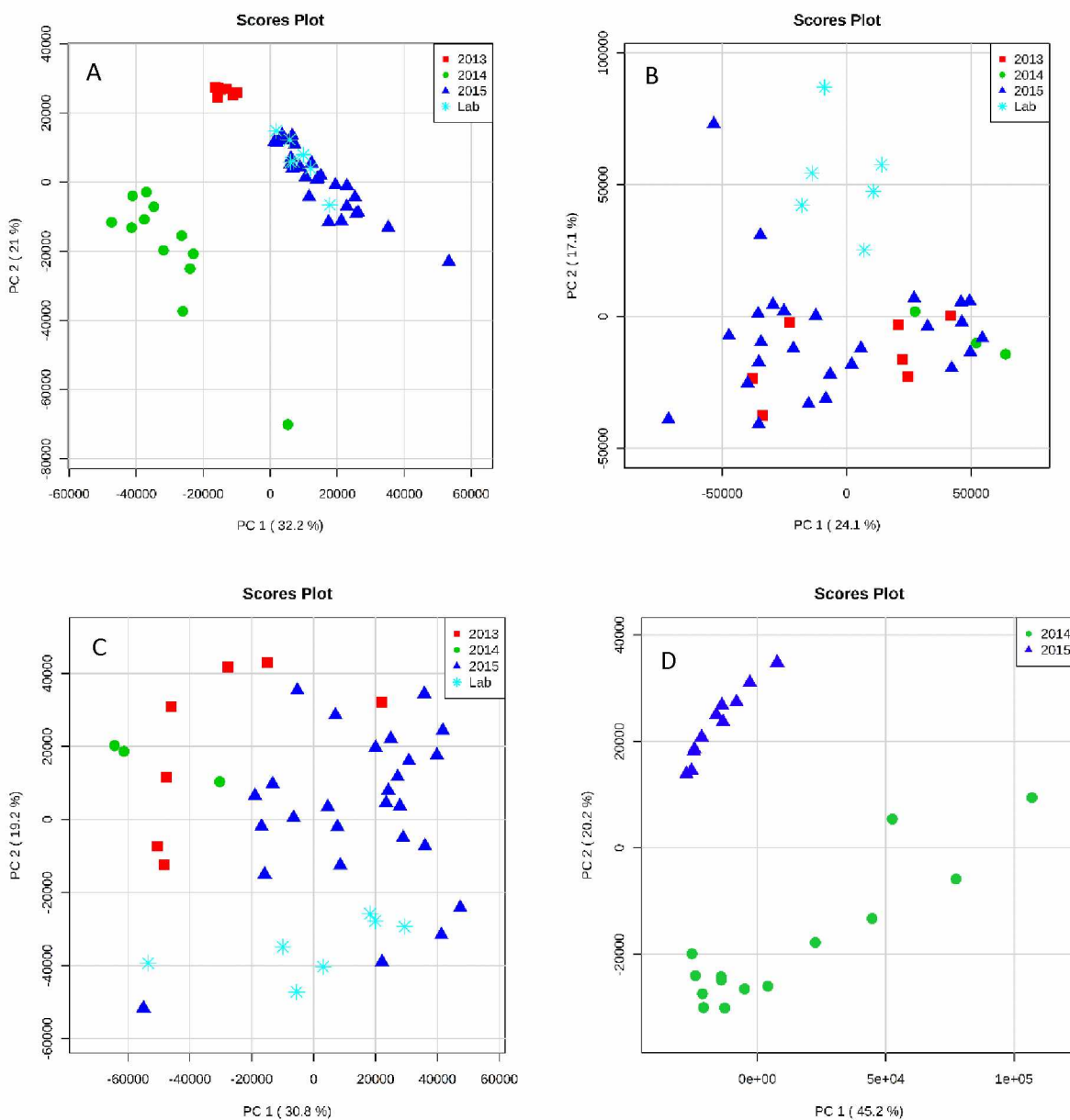


Figure 3.3. Principle components analysis score plots for uniquely identified features (potential metabolites) in A) *P. camtschaticus* hemolymph, B) *P. camtschaticus* hepatopancreas [note: 2013 was visually separated from others in PC 3], C) *P. camtschaticus* muscle, and D) *L. aequispinus* hemolymph. Score plot symbols show crab collected in 2013, 2014, 2015, and crab kept in a laboratory for one year and sampled in 2015.

model ($R^2 = 0.31$, $Q^2 = 0.13$; Fig. 3.4), with separation occurring primarily between the sexes.

Using univariate analyses for hemolymph, no differences were found in *P. camtschaticus*, while one feature differed in *L. aequispinus*, with a higher concentration found in healthy females (Table 3.2). In *P. camtschaticus* hepatopancreas, three features differed, principally between the sexes (Table 3.2). The *P. camtschaticus* muscle samples had the highest number of significant features (19), with most differences occurring between the sexes, but with some apparent interactions between sex and infection state (Table 3.2). In no case did metabolites vary with infection state, irrespective of sex.

The molecular formulae and putative identities (level 3; Sumner *et al.* 2007) of significant features and their possible functions are listed in Table 2. Sixty-one percent of significant features had a METLIN database match to one or more compounds (i.e., isomers).

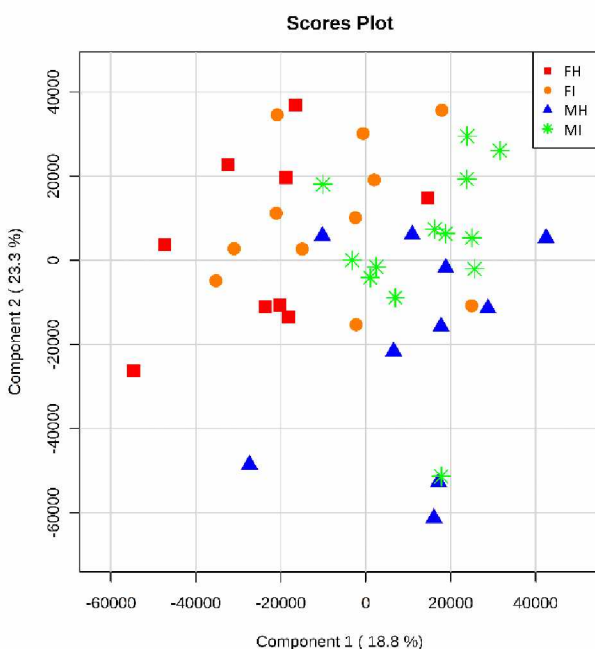


Figure 3.4. Partial Least Squares Discriminant Analysis (PLS-DA) plots for uniquely identified features in *Paralithodes camtschaticus* muscle (leave-one-out cross validation: $R^2 = 0.31$, $Q^2 = 0.13$; permutation test: $P = 0.018$). Score plot symbols show healthy females (FH), infected females (FI), healthy males (MH), and infected males (MI).

Table 3.2. Molecular formulae and putative identities of metabolites that differed among *Paralithodes camtschaticus* and *Lithodes aequispinus* sexes and infection states (with or without infection by *Briarosaccus*). For each feature, neutral mass (mDa), retention time (RT; minutes), mass error (ppm), p-value (with False Discovery Rate applied), Tukey post-hoc tests (FH = female healthy, FI = female infected, MH = male healthy, MI = male infected), and putative identities are given.

Molecular Formula	Neutral Mass	RT	Mass Error	P	Post-Hoc Tests	Putative ID – Description *^
<i>Lithodes aequispinus</i> hemolymph						
C ₉ H ₁₁ NO ₆ S	261.0304	2.693	1.18	0.004	FH > FI MH MI	No ID
<i>Paralithodes camtschaticus</i> hepatopancreas						
C ₁₂ H ₁₈	162.1402	2.024	3.94	0.020	FH > MH FI > MH MI	No ID
C ₁₂ H ₂₂ O ₂	198.1618	2.022	0.77	0.030	FH FI > MH MI	5-dodecenoic acid – <i>saturated fatty acid with antimicrobial properties</i> (Kitahara <i>et al.</i> , 2004)
C ₁₂ H ₂₀ O	180.1505	2.025	5.08	0.020	FH FI > MH MI	[9,11/8,10/5,7/7,9]-Dodecadialenal – <i>sex attractant for some Lepidoptera</i> (Chisholm & Reed, 1985) [3,6,8/3,6,9]-Dodecatrienol – <i>sex & trail following pheromone in Isoptera</i> (Saran <i>et al.</i> , 2007; Sillam-Dussès <i>et al.</i> , 2011)
<i>Paralithodes camtschaticus</i> muscle						
C ₂₃ H ₂₅ N ₃ O ₂₅	743.0757	1.994	2.73	0.030	MH > FH MI > FH FI	No ID
C ₁₄ H ₄₁ N ₁₆ O ₃	481.3548	5.957	-0.17	0.047	FH > MH MI	No ID
C ₁₅ H ₂₁ N ₃ O ₉ S	419.0995	4.911	0.82	0.040	MH MI > FH	No ID
C ₂₅ H ₃₃ N ₃ O ₁₃ S	615.1728	0.597	1.03	0.028	MH MI > FH FI	No ID
C ₄ H ₅ N ₃ O ₃ S	175.0041	0.827	6.19	0.028	FI > FH MH MI	No ID
C ₃₅ H ₆₄ O ₄	548.4805	9.010	-0.01	0.028	FH > MH MI	No ID
C ₄₇ H ₇₀ N ₈ O	702.5668	9.244	0.62	0.028	FH > FI MI MH	No ID
C ₈ H ₁₃ NO ₄	187.0851	10.604	-3.39	0.030	MH > FH FI MI > FI	6-Acetamido-3-oxohexanoate – <i>lysine degradation</i> (KEGG - Kanehisa & Goto, 2000) N2-Acetyl-aminoadipate semialdehyde – <i>involved in prokaryote lysine synthesis pathway</i> (Cerqueira <i>et al.</i> , 2014) N-(hydroxy-butanoyl)-homoserine lactone – <i>luminescence & virulence of Vibrio harveyi</i> (Manefield <i>et al.</i> , 2000)
C ₁₇ H ₃₁ N ₃ O ₄	341.2300	1.577	4.20	0.028	MH MI > FH FI	Pro [Ile/Leu] [Ile/Leu] – <i>Diprotin A (Ile Pro Ile), inhibitor insect dipeptidyl peptidase</i> (Rahfeld <i>et al.</i> , 1991; Nässel <i>et al.</i> , 2000)
C ₁₄ H ₁₉ NO ₄	265.1316	8.329	-0.54	0.028	FH > MI MH	Anisomycin – <i>Antibiotic produced by Streptomyces, inhibits protein synthesis</i> (PubChem - Kim <i>et al.</i> , 2016)
C ₅ H ₁₁ NO ₂	117.0801	0.970	9.79	0.031	MH > FH FI	N(alpha)-Benzoyloxycarbonyl-L-leucine – <i>leucine derivative</i> [4/5/2]-aminopentanoic acid – <i>lysine degradation, high levels indicate tissue necrosis/ bacteria</i> (PubChem - Kim <i>et al.</i> , 2016) Valine – <i>amino acid, crab molting change</i> (Wheatly, 1985) 4-Methylaminobutyrate – <i>microbial metabolism</i> (KEGG - Kanehisa & Goto, 2000)
C ₈ H ₁₂ O ₃	156.0793	5.722	-3.92	0.028	FI > MI MH	5-oxo-7-octenoic acid – <i>fatty acid with potential antifungal properties</i> (Abdullah <i>et al.</i> , 2014)
C ₁₆ H ₂₅ N ₅ O ₆	383.1809	4.589	-1.03	0.047	FI > MH FH	His [Ile/Leu/Val] [Asp/Glu] – <i>tripeptide, histidine peptides can have antioxidative properties</i> (Sarmadi & Ismail, 2010)
C ₃₃ H ₅₄ N ₇ O ₆	546.3954	8.470	-6.23	0.028	FI FH > MI MH	Cholest-5,24-dien-3beta-yl beta-D-glucopyranoside – <i>a cholesterol</i>
C ₃₈ H ₇₇ N ₂ O ₆ P	688.5502	8.954	2.5	0.020	FH > MH MI FI > MI	Etn-1-P-Cer(d14:1/18:0) – <i>sphingolipid (signal transmission, cell recognition)</i>
C ₇ H ₇ NO ₂	137.0482	0.675	-3.61	0.040	FI > MI MH FH	[2/3/4]-Aminobenzoic acid – <i>intermediate in folate synthesis in plants, bacteria, & fungi</i> (Riedlinger <i>et al.</i> , 2004)
C ₃₃ H ₅₄ O ₅	530.4009	8.480	-7.09	0.303	FH > MH MI	Vitamin E succinate – <i>antioxidant</i>
C ₁₉ H ₂₂ FN ₃ O ₂	327.1759	8.481	-3.54	0.028	FH > MH MI	*Azaperone – <i>veterinary tranquilizer, detected in oysters from the Mediterranean Sea</i> (Alvarez-Muñoz <i>et al.</i> , 2015)
C ₂₃ H ₁₈ O ₇	406.1061	10.598	-2.06	0.047	MH > FH FI MI > FI	*Rotenone – <i>Flavonoid, breakdown product of rotenone (pesticide, used commercially)</i> (PubChem - Kim <i>et al.</i> , 2016)

* Part of compounds in brackets indicate that it could be any of possibilities listed within the brackets (separated by backslashes).

^ Tripeptides could be connected in any order

+ Potential contaminates

Two of the putative identities, rotenonone (breakdown product of the pesticide rotenone) and azaperone (veterinary tranquilizer), are not biologically relevant compounds for king crab. These compounds could be misidentified, or the compounds could have been taken up by crab from environmental sources. For the Juneau sample collection site, runoff, discharge from sewage treatment facilities, fisheries, and cruise ships are all possible sources of contaminants. To investigate the possibility of a regional effect on metabolites, the collection locations for the crab with the 10 highest values for these two contaminants were mapped to identify possible point sources (Fig. 3.5). Crab with the highest amounts of rotenonone tended to cluster around Auke Bay, Alaska, while crab with the highest abundance of azaperone did not appear clustered around a specific location (Fig. 3.5).

Because collection year had a strong effect on the variation in metabolites, it may have masked metabolite changes that occur with parasite infection. To determine if we could detect metabolite changes with infection state within a year (sexes combined), we reran multivariate and univariate analyses on *P. camtschaticus* caught in Juneau, Alaska in a single year (2015, which had the largest sample size). In univariate analyses, there were no significant features in the hemolymph or hepatopancreas and one unidentifiable feature in muscle: $C_{10}H_{16}N_2O_3$ (m/z 212.1175 @ 0.833 min). Multivariate analyses did not show any significant clustering (PLS-DA: $P > 0.1$). To identify any other confounding variables that could still be masking the effects of infection condition, for 2015, PCAs were run using local-scale sample collection locations as classifiers. For hemolymph and hepatopancreas, there was no apparent clustering (not shown); however, collection location was important in distinguishing muscle samples, with samples collected in Auke Bay and the surrounding area differing from other collection locations (Fig. 3.6).

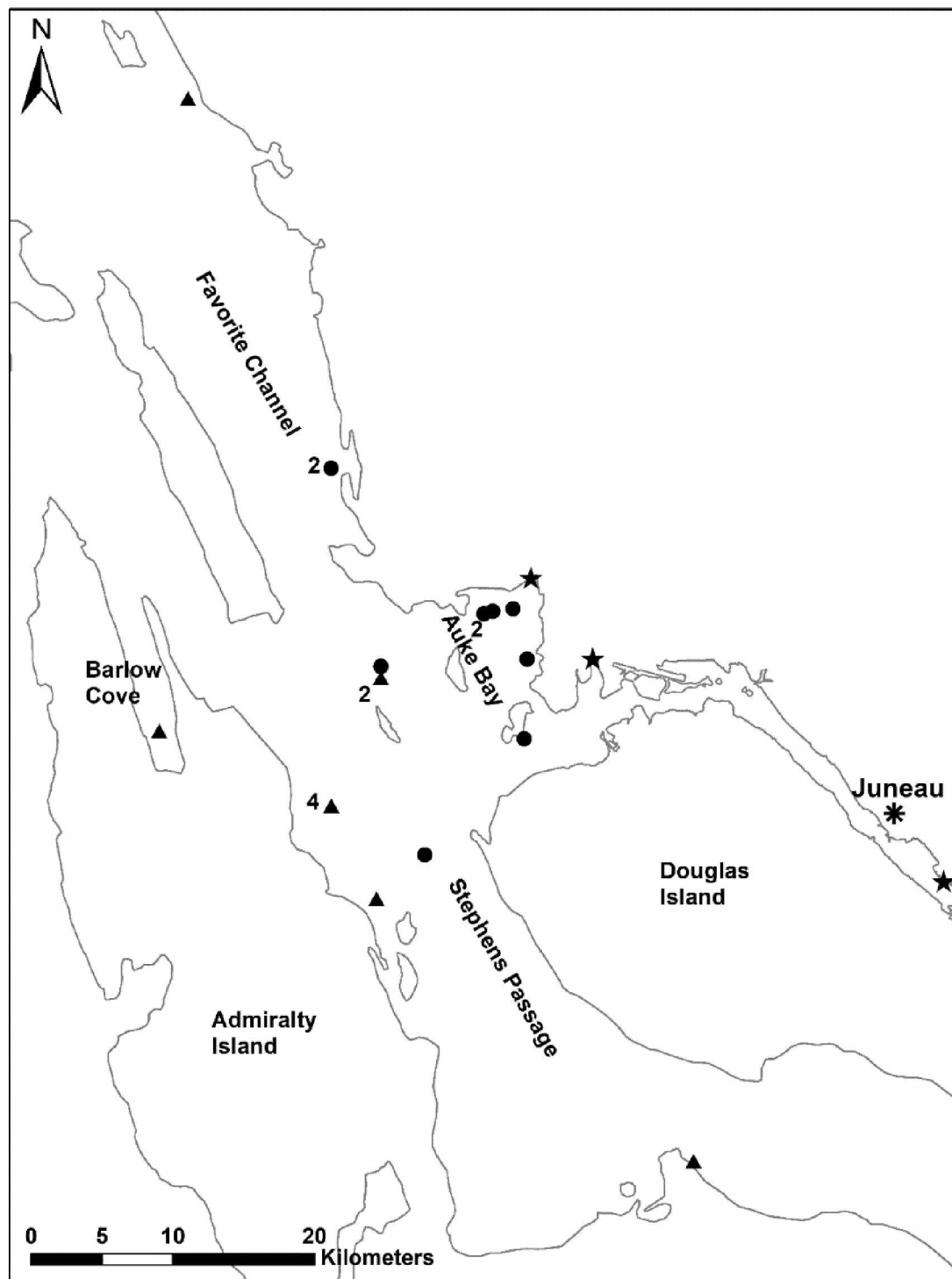


Figure 3.5. Capture locations of *Paralithodes camtschaticus* with the 10 highest values for two putatively identified contaminants, azaperone (triangles) and rotenonone (circles). Stars indicate areas where sewage treatment facilities discharge wastewater, a potential source of these marine contaminants. When symbols overlap, numbers indicate the number of crab represented by that symbol.

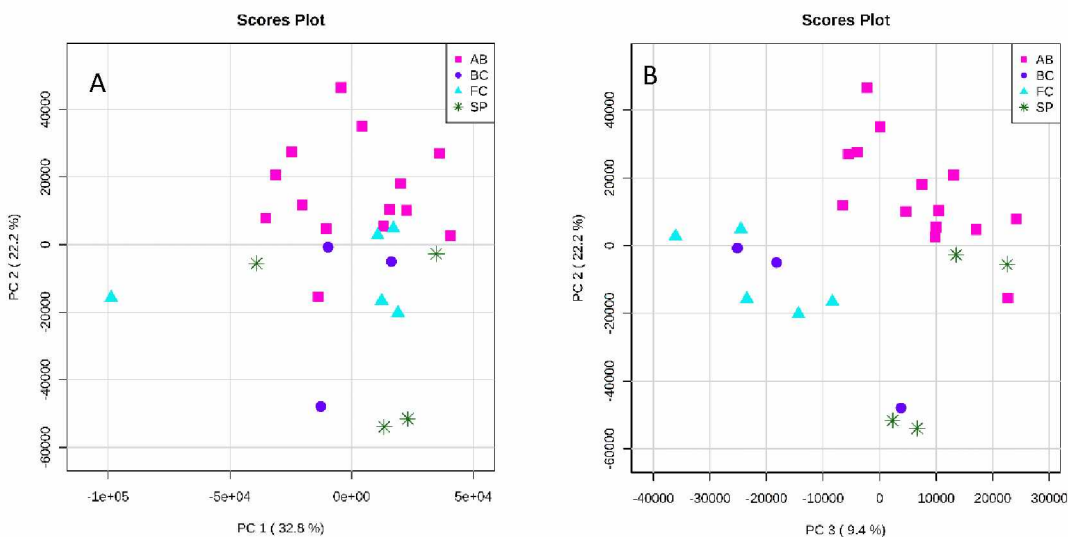


Figure 3.6. Principle components analysis of putative metabolites identified from *Paralithodes camtschaticus* muscle from crab sampled in 2015 around Juneau, Alaska. Symbols are coded based on collection location (AB – Auke Bay; BC – Barlow Cove; FC – Favorite Channel; SP – Stephens Passage). A) principal component (PC) 1 versus PC2 and B) PC2 versus PC3. The clustering of Auke Bay samples indicates that the metabolome of crab caught in Auke Bay differed from other nearby locations.

Discussion

We investigated variations in the metabolome of male and female *P. camtschaticus* and *L. aequispinus* infected by *Briarosaccus* spp. Metabolomic analyses were conducted using muscle, hemolymph, and hepatopancreas from king crab collected in the field from multiple years and locations. Sample location and year (Figs. 3.3 and 3.6) produced distinct metabolite profiles, while the metabolite profiles varied little with crab sex and infection state (Fig. 3.2). Unique features that distinguished the sexes and infection states were found primarily within muscle samples; most features differed between the sexes, while a few showed an interaction between sex and infection state (Table 3.2).

This study was the first to use metabolomic analysis in a rhizocephalan and host crab parasitic relationship. Metabolomics have been used to quantify the effects of other stressors in

decapods (Schock *et al.*, 2010; Hammer *et al.*, 2012), but these studies used nuclear magnetic resonance (NMR) based metabolomics. NMR has a lower capacity for identifying rare features, although feature identification tends to be easier and NMR can be used to identify unknowns (Markley *et al.*, 2017). LC-MS based metabolomics has been applied in decapods to distinguish crab species (Laith *et al.*, 2017), and in other crustaceans to examine how the metabolome changes with starvation, pollutants, and environment (Ralston-Hooper *et al.*, 2011; Maity *et al.*, 2012, 2013). However, studies of metabolites in crustaceans have mostly been conducted in laboratory settings, which exclude environmental variability and focus on a single stressor. In this study, multivariate analyses could not distinguish between healthy crab and those infected with *Briarosaccus*, yet this marks one of the first attempts to look for metabolite variation in crustaceans, while including natural environmental variability (i.e., field collected samples) across space and time. Similar to our results, effects of a bacterial (*Vibrio campbellii* [Baumann, Baumann & Mandel, 1971] Baumann, Baumann, Bang & Woolkalis, 1981) infection could not be detected in the metabolome of field collected *Callinectes sapidus* Rathbun, 1896 (blue crab) using NMR-based metabolomics (Schock *et al.*, 2010). In contrast, laboratory trials produced a detectable response when crab were injected with *V. campbellii* and monitored over a short time interval (4 hours) (Schock *et al.*, 2010). When exposed to any sort of change or perturbation, the metabolites in a cell can respond very quickly, changing in a manner of seconds to minutes (Link *et al.*, 2015; Fessenden, 2016). Thus, when working with samples from a natural environment, it can be difficult to detect consistent signals from a single stressor, like parasitism, owing to the noise caused by changes in numerous other factors (e.g., food intake, amount of movement, temperature).

Despite environmental variability, five features showed an interaction between sex and *Briarosaccus* parasitism, i.e., the effect of parasite infection on the relative amounts of these compounds in the metabolome differed between the sexes. Two of the unidentified features ($C_9H_{11}NO_6S$ in *L. aequispinus* hemolymph and $C_{42}H_{70}N_8O$ in *P. camtschaticus* muscle; Table 3.2) were upregulated in healthy female crab. These features could potentially be involved in the female reproductive cycle (e.g., vitellogenesis inhibiting hormone or crustacean hyperglycemic hormone pathways; Swetha *et al.*, 2011), because they would be depressed in the infected, sterilized females. In contrast, three features, an aminobenzoic acid, a histidine tripeptide, and the unidentified feature $C_4H_5N_3O_3S$, were upregulated in the muscle of infected *P. camtschaticus* females. If rhizocephalans respond differently depending on host sex, then these features in female hosts could be derived from or induced by the parasite. Alternatively, female and male crab may have different responses to infection, as they do with other stressors, like oxidative stress (Paital & Chainy, 2013). The differential expression of four of these five metabolites in the crab muscle is of particular interest because the rootlets of *Briarosaccus* do not penetrate the leg muscles (Noever *et al.*, 2016). Rhizocephalans can cause changes in tissues, like gonads, without direct rootlet penetration (Rubiliani & Godette, 1981; Rubiliani, 1985; Høeg, 1995); however, the reason why these signals were not found in the hemolymph or hepatopancreas is interesting. Muscle tissue has slower protein synthesis and turnover than hemolymph and hepatopancreas (Bodin *et al.*, 2007; deVries *et al.*, 2015). Thus, muscle metabolites may be more stable, allowing for the detection of signals from the parasite, despite environmental variability.

One of the five features with an interaction between infection status and sex was an aminobenzoic acid, with elevated amounts in muscle of infected female crab. 4-aminobenzoic

acid is an intermediate in the synthesis of the vitamin folate (Brown, 1962) in plants, bacteria, and fungi; although animals cannot synthesize folate, their gut bacteria can (Camilo *et al.*, 1996). The open circulatory system in crustaceans may have allowed 4-aminobenzoic acid that was produced in the crab gut by bacteria to reach the muscle. Folic acid is important in the diet of Chinese mitten crab (*Eriocheir sinensis* H. Milne Edwards, 1853), because it can increase the innate immune response and relieve oxidative stress (Wei *et al.*, 2016). It is difficult to explain why folate synthesis by gut bacteria or uptake from dietary sources would increase in infected female crab, but location-specific changes in a folate degradation product have been detected in other crustaceans (Maity *et al.*, 2013).

Tripeptides are important in distinguishing the metabolomes of brachyuran crab species (Laith *et al.*, 2017). Here, the histidine tripeptide, with a branched-chain amino acid (leucine, isoleucine, or valine) and an acidic amino acid (aspartate or glutamate), was upregulated in the muscle of infected female *P. camtschaticus*. Histidine peptides are associated with antioxidant properties (Sarmadi & Ismail, 2010). Abiotic stressors, like temperature, salinity, and dissolved oxygen content, affect the levels of other antioxidants in some decapods (Paital & Chainy, 2013), thus it is possible that this peptide is upregulated by the host crab as a response to parasitic stress. In addition, aspartate and glutamate are involved in neurotransmission in crustaceans (Crawford & McBurney, 1977), which could be affected by parasite infection. Host muscle infiltrated by rootlets of *Sacculina carcini* Thompson, 1836 lost fibrillar protein (Powell & Rowley, 2008), which the authors attributed to mobilization of muscle proteins by the rhizocephalan. The histidine tripeptide could have been produced via muscle protein catabolism to produce more energy for the interna. Overall, there are multiple possible explanations for an increase in the

histidine metabolite in infected female crab, but why a similar response is not seen in males deserves further scrutiny.

Conclusions

This study highlights the variability of the crustacean metabolome with spatial and temporal variation. Many metabolites have multiple functions and pathways, which complicates interpretation (Prosser *et al.*, 2014). We only found metabolites to differ by infection state when male and female crabs were examined separately, suggesting that *Briarosaccus* may have different effects on the metabolomes of each sex.

A larger sample size or less environmental variability may have produced more differential metabolites between the sexes and infection states. Here the sample size ($n = 4 - 15$, depending on condition; Table 3.1) was comparable to other environmental metabolomics studies with marine invertebrates (e.g., $n = 3 - 14$; Rosenblum *et al.*, 2005, Hines *et al.*, 2007, Schock *et al.*, 2010, Ralston-Hooper *et al.*, 2011, Laith *et al.*, 2017), yet it may not have been sufficient to detect signals across the environmental variability included (Lin *et al.*, 2017). For example, metabolomics studies of human disease can require hundreds of samples to fully encompass the variability of the metabolome (Mamas *et al.*, 2011; Dunn *et al.*, 2014). Large sample sizes are not always feasible, especially with a rare parasite, like *Briarosaccus*, on a subtidal host. Instead, decreasing the included environmental variation, so as to necessitate a lower sample size, could increase differential metabolite detection. If crab were held in a laboratory under constant conditions, this could potentially stabilize the metabolome, allowing for signals from the parasite to be detected. However, a study with mussels (*Mytilus galloprovincialis* Lamarck, 1819) found that field sampled organisms had lower variability in the metabolome than those “stabilized” under laboratory conditions (Hines *et al.*, 2007). To further

unravel how *Briarosaccus* and other rhizocephalans affect the metabolome of their host crab, targeted metabolomics could be used to focus on specific compounds that are likely to be affected, like those involved with sex determination, molting, reproduction, and growth.

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References

- Abdullah, S., Gobilik, J. and Chong, K.P. 2014. Antifungal phytochemical compounds of *Cynodon dactylon* and their effects on *Ganoderma boninense*. *American-Eurasian Journal of Sustainable Agriculture*, **8**: 22–27.
- Alvarez-Muñoz, D., Huerta, B., Fernandez-Tejedor, M., Rodríguez-Mozaz, S. and Barceló, D. 2015. Multi-residue method for the analysis of pharmaceuticals and some of their metabolites in bivalves. *Talanta*, **136**: 174–182.

- Barnes, S., Benton, H.P., Casazza, K., Cooper, S.J., Cui, X., Du, X., Engler, J., Kabarowski, J.H., Li, S., Pathmasiri, W., Prasain, J.K., Renfrow, M.B. and Tiwari, H.K. 2016. Training in metabolomics research. II. Processing and statistical analysis of metabolomics data, metabolite identification, pathway analysis, applications of metabolomics and its future. *Journal of Mass Spectrometry*, **51**: 535–548.
- Benjamini, Y. and Hochberg, Y. 1995. Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society B*, **57**: 289–300.
- Bodin, N., Le Loc'h, F. and Hily, C. 2007. Effect of lipid removal on carbon and nitrogen stable isotope ratios in crustacean tissues. *Journal of Experimental Marine Biology and Ecology*, **341**: 168–175.
- Bresciani, J. and Høeg, J.T. 2001. Comparative ultrastructure of the root system in rhizocephalan barnacles (Crustacea: Cirripedia: Rhizocephala). *Journal of Morphology*, **249**: 9–42.
- Brown, G.M. 1962. The biosynthesis of folic acid. II. Inhibition by sulfonamides. *The Journal of Biological Chemistry*, **237**: 536–540.
- Camilo, E., Zimmerman, J., Mason, J.B., Golner, B., Russell, R., Selhub, J. and Rosenberg, I.H. 1996. Folate synthesized by bacteria in the human upper small intestine is assimilated by the host. *Gastroenterology*, **110**: 991–998.
- Cerqueira, G.C., Arnaud, M.B., Inglis, D.O., Skrzypek, M.S., Binkley, G., Simison, M., Miyasato, S.R., Binkley, J., Orvis, J., Shah, P., Wymore, F., Sherlock, G. and Wortman, J.R. 2014. The *Aspergillus* genome database: Multispecies curation and incorporation of RNA-Seq data to improve structural gene annotations. *Nucleic Acids Research*, **42**: 705–710.

- Chisholm, M.D. and Reed, D.W. 1985. 9,11-Dodecadienyl alcohols, acetates, or aldehydes as synthetic chemical sex attractants for four Lepidoptera: *Cosmopterix gemmiferella* (Clemens), *Dichrorampha simulana* (Clemens), *Tortricidia testacea* (Packard), and an *Ancyliis*. *Journal of Chemical Ecology*, **11**: 1341–1347.
- Crawford, A.C. and McBurney, R.N. 1977. The synergistic action of L-glutamate and L-aspartate at crustacean excitatory neuromuscular junctions. *Journal of Physiology*, **268**: 697–709.
- deVries, M.S., Rittschof, D. and Forward, R. 1989. Response by rhizocephalan-parasitized crabs to analogues of crab larval-release pheromones. *Journal of Crustacean Biology*, **9**: 517–524.
- deVries, M.S., del Rio, C.M., Tunstall, T.S. and Dawson, T.E. 2015. Isotopic incorporation rates and discrimination factors in mantis shrimp crustaceans. *PLOS ONE*, **10**: e0122334.
- Dunn, W.B., Lin, W., Broadhurst, D., Begley, P., Brown, M., Zelena, E., Vaughan, A.A., Halsall, A., Harding, N., Knowles, J.D., Francis-McIntyre, S., Tseng, A., Ellis, D.I., O'Hagan, S., Aarons, G., Benjamin, B., Chew-Graham, S., Moseley, C., Potter, P., Winder, C.L., Potts, C., Thornton, P., McWhirter, C., Zubair, M., Pan, M., Burns, A., Cruickshank, J.K., Jayson, G.C., Purandare, N., Wu, F.C.W., Finn, J.D., Haselden, J.N., Nicholls, A.W., Wilson, I.D., Goodacre, R. and Kell, D.B. 2014. Molecular phenotyping of a UK population: Defining the human serum metabolome. *Metabolomics*, **11**: 9–26.
- ESRI. 2014. ArcGIS Desktop: Release 10.3. Environmental Systems Research Institute, Redlands, CA.
- Fessenden, M. 2016. Metabolomics: Small molecules, single cells. *Nature*, **540**: 153–155.

- Glenner, H. 2001. Cypris metamorphosis, injection and earliest internal development of the Rhizocephalan *Loxothylacus panopaei* (Gissler). Crustacea: Cirripedia: Rhizocephala: Sacculinidae. *Journal of Morphology*, **249**: 43–75.
- Hammer, K.M., Pedersen, S.A. and Storseth, T.R. 2012. Elevated seawater levels of CO₂ change the metabolic fingerprint of tissues and hemolymph from the green shore crab *Carcinus maenas*. *Comparative Biochemistry and Physiology - Part D: Genomics and Proteomics*, **7**: 292–302.
- Hawkes, C.R., Meyers, T.R. and Shirley, T.C. 1986. Prevalence of the parasitic barnacle *Briarosaccus callosus* on king crabs of southeastern Alaska. *Transactions of the American Fisheries Society*, **115**: 252–257.
- Hines, A., Oladiran, G.S., Bignell, J.P., Stentiford, G.D. and Viant, M.R. 2007. Direct sampling of organisms from the field and knowledge of their phenotype: Key recommendations for environmental metabolomics. *Environmental Science and Technology*, **41**: 3375–3381.
- Høeg, J.T. 1995. The biology and the life-cycle of the rhizocephala (Cirripedia). *Journal of the Marine Biological Association of the United Kingdom*, **75**: 517–550.
- Høeg, J.T. and Lützen, J. 1995. Life cycle and reproduction in the Cirripedia Rhizocephala. *Oceanography and Marine Biology: An Annual Review*, **33**: 427–485.
- Høeg, J.T. and Møller, O.S. 2006. When similar beginnings lead to different ends: Constraints and diversity in cirripede larval development. *Invertebrate Reproduction and Development*, **49**: 125–142.

- Hsiao, C.J., Wu, Y.I., Tung, T.A., Wang, G.Y., Toullec, J.Y., Liu, S.T., Huang, W.S. and Lee, C.Y. 2016. Metabolic effects of parasitization by the barnacle *Polyascus plana* (Cirripedia: Rhizocephala: Sacculinidae) on a grapsid host, *Metopograpsus thukuhar*. *Diseases of Aquatic Organisms*, **119**: 199–206.
- Isaeva, V., Dolganov, S. and Shukalyuk, A. 2005. Rhizocephalan barnacles—parasites of commercially important crabs and other decapods. *Russian Journal of Marine Biology*, **31**: 215–220.
- Kaddurah-Daouk, R., Kristal, B.S. and Weinshilboum, R.M. 2008. Metabolomics: a global biochemical approach to drug response and disease. *Annual Review of Pharmacology and Toxicology*, **48**: 653–683.
- Kafsack, B.F.C. and Llinás, M. 2010. Eating at the table of another: Metabolomics of host-parasite interactions. *Cell host and Microbe*, **7**: 90–99.
- Kanehisa, M. and Goto, S. 2000. Kyoto encyclopedia of genes and genomes. *Nucleic Acids Research*, **28**: 27–30.
- Kim, S., Thiessen, P.A., Bolton, E.E., Chen, J., Fu, G., Gindulyte, A., Han, L., He, J., He, S., Shoemaker, B.A., Wang, J., Yu, B., Zhang, J. and Bryant, S.H. 2016. PubChem substance and compound databases. *Nucleic Acids Research*, **44**: D1202–D1213.
- Kitahara, T., Koyama, N., Matsuda, J., Aoyama, Y., Hirakata, Y., Kamihira, S., Kohno, S., Nakashima, M. and Sasaki, H. 2004. Antimicrobial activity of saturated fatty acids and fatty amines against methicillin-resistant *Staphylococcus aureus*. *Biological and Pharmaceutical Bulletin*, **27**: 1321–1326.

- Laith, A.A., Ambak, M., Abol-Munafi, A.B., Nurhafizah, W.W.I. and Najiah, M. 2017. Metabolomic analysis of marine and mud crabs based on antibacterial activity. *Aquaculture Reports*, **7**: 7–15.
- Lankadurai, B.P., Nagato, E.G. and Simpson, M.J. 2013. Environmental metabolomics: An emerging approach to study organism responses to environmental stressors. *Environmental Reviews*, **21**: 180–205.
- Lin, Y.S., Shireman, L. and McFeely, S. 2017. Metabolomics approaches in toxicology, Volume 1: General Principles. In: *Comprehensive Toxicology* (C. McQueen, ed), pp. 391–414. Elsevier Science, Amsterdam.
- Lindon, J.C., Nicholson, J.K. and Holmes, E. 2007. *The Handbook of Metabonomics and Metabolomics*. Elsevier Science, Amsterdam.
- Link, H., Fuhrer, T., Gerosa, L., Zamboni, N. and Sauer, U. 2015. Real-time metabolome profiling of the metabolic switch between starvation and growth. *Nature Methods*, **12**: 1091–1097.
- Maity, S., Jannasch, A., Adamec, J., Gribskov, M., Nalepa, T., Höök, T.O. and Sepúlveda, M.S. 2012. Metabolite profiles in starved *Diporeia* spp. using liquid chromatography - mass spectrometry (LC-MS) based metabolomics. *Journal of Crustacean Biology*, **32**: 239–248.
- Maity, S., Jannasch, A., Adamec, J., Watkins, J.M., Nalepa, T., Höök, T.O. and Sepúlveda, M.S. 2013. Elucidating causes of *Diporeia* decline in the Great Lakes via metabolomics: physiological responses after exposure to different stressors. *Physiological and Biochemical Zoology*, **86**: 213–223.

- Mamas, M., Dunn, W.B., Neyses, L. and Goodacre, R. 2011. The role of metabolites and metabolomics in clinically applicable biomarkers of disease. *Archives of Toxicology*, **85**: 5–17.
- Manefield, M., Harris, L., Rice, S.A., De Nys, R. and Kjelleberg, S. 2000. Inhibition of luminescence and virulence in the black tiger prawn (*Penaeus monodon*) pathogen *Vibrio harveyi* by intercellular signal antagonists. *Applied and Environmental Microbiology*, **66**: 2079–2084.
- Markley, J.L., Brüschweiler, R., Edison, A.S., Eghbalian, H.R., Powers, R., Raftery, D. and Wishart, D.S. 2017. The future of NMR-based metabolomics. *Current Opinion in Biotechnology*, **43**: 34–40.
- Nässel, D.R., Mentlein, R., Bollner, T. and Karlsson, A. 2000. Proline-specific dipeptidyl peptidase activity in the cockroach brain and intestine: Partial characterization, distribution, and inactivation of tachykinin-related peptides. *Journal of Comparative Neurology*, **418**: 81–92.
- Noever, C., Keiler, J. and Glenner, H. 2016. First 3D reconstruction of the rhizocephalan root system using MicroCT. *Journal of Sea Research*, **113**: 58–64.
- Orensanz, J.M., Armstrong, J., Armstrong, D. and Hilborn, R. 1998. Crustacean resources are vulnerable to serial depletion – the multifaceted decline of crab and shrimp fisheries in the greater Gulf of Alaska. *Reviews in Fish Biology and Fisheries*, **176**: 117–176.
- Oug, E., Cochrane, S.K.J., Sundet, J.H., Norling, K. and Nilsson, H.C. 2011. Effects of the invasive red king crab (*Paralithodes camtschaticus*) on soft-bottom fauna in Varangerfjorden, northern Norway. *Marine Biodiversity*, **41**: 467–479.

- Paital, B. and Chainy, G.B.N. 2013. Seasonal variability of antioxidant biomarkers in mud crabs (*Scylla serrata*). *Ecotoxicology and Environmental Safety*, **87**: 33–41.
- Payen, G.G., Rubiliani, C., Hubert, M., Poulhe, R., Turquier, Y. and Chassard-Bouchaud, C. 1983. Mise en evidence des fonctions de synthese, d'absorption et de transfert de substances par les racines des Rhizocephales Sacculinidae; premieres donnees biochimiques et cytochimiques. *Zeitschrift fur Parasitenkunde*, **69**: 255–269.
- Powell, A. and Rowley, A.F. 2008. Tissue changes in the shore crab *Carcinus maenas* as a result of infection by the parasitic barnacle *Sacculina carcini*. *Diseases of Aquatic Organisms*, **80**: 75–79.
- Poynton, H.C., Taylor, N.S., Hicks, J., Colson, K., Chan, S., Clark, C., Scanlan, L., Loguinov, A. V., Vulpe, C. and Viant, M.R. 2011. Metabolomics of microliter hemolymph samples enables an improved understanding of the combined metabolic and transcriptional responses of *Daphnia magna* to cadmium. *Environmental Science and Technology*, **45**: 3710–3717.
- Prosser, G.A., Larrouy-Maumus, G. and de Carvalho, L.P.S. 2014. Metabolomic strategies for the identification of new enzyme functions and metabolic pathways. *EMBO Reports*, **15**: 657–669.
- Rahfeld, J., Schierborn, M., Hartrodt, B., Neubert, K. and Heins, J. 1991. Are diprotin A (Ile-Pro-Ile) and diprotin B (Val-Pro-Leu) inhibitors or substrates of dipeptidyl peptidase IV? *Biochimica et Biophysica Acta (BBA)/Protein Structure and Molecular*, **1076**: 314–316.
- Ralston-Hooper, K.J., Adamec, J., Jannash, A., Mollenhauer, R., Ochoa-Acuña, H. and Sepúlveda, M.S. 2011. Use of GC × GC/TOF-MS and LC/TOF-MS for metabolomic analysis of *Hyalella azteca* chronically exposed to atrazine and its primary metabolite, desethylatrazine. *Journal of Applied Toxicology*, **31**: 399–410.

- Reinhard, E. 1956. Parasitic castration of Crustacea. *Experimental Parasitology*, **5**: 79–107.
- Riedlinger, J., Reichke, A., Zähler, H., Krismer, B., Bull, A.T., Maldonado, L.A., Ward, A.C., Goodfellow, M., Bister, B., Bischoff, D., Süssmuth, R.D. and Fiedler, H. 2004. Abyssomicins, inhibitors of the para-aminobenzoic acid pathway produced by the marine *Verrucosipora* strain AB-18-032. *The Journal of Antibiotics*, **57**: 271–279.
- Ritchie, L. and Høeg, J.T. 1981. The life history of *Lernaodiscus porcellanae* (Cirripedia: Rhizocephala) and co-evolution with its porcellanid hosts. *Journal of Crustacean Biology*, **1**: 334–347.
- Rosenblum, E.S., Viant, M.R., Braid, B.M., Moore, J.D., Friedman, C.S. and Tjeerdema, R.S. 2005. Characterizing the metabolic actions of natural stresses in the California red abalone, *Haliotis rufescens* using ¹H NMR metabolomics. *Metabolomics*, **1**: 199–209.
- Rubiliani, C. 1985. Response by two species of crabs to a rhizocephalan extract. *Journal of Invertebrate Pathology*, **45**: 304–310.
- Rubiliani, C. and Godette, G.O. 1981. Radiochemical and electrophoretic studies on the CNS of crabs parasitized with rhizocephalans: Comparison with healthy crabs. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, **70**: 415–419.
- Sana, T.R., Gordon, D.B., Fischer, S.M., Tichy, S.E., Kitagawa, N., Lai, C., Gosnell, W.L. and Chang, S.P. 2013. Global mass spectrometry based metabolomics profiling of erythrocytes infected with *Plasmodium falciparum*. *PLOS ONE*, **8**: e60840.
- Sana, T.R., Roark, J.C., Li, X., Waddell, K. and Fischer, S.M. 2008. Molecular formula and METLIN personal metabolite database matching applied to the identification of compounds generated by LC/TOF-MS. *Journal of Biomolecular Techniques*, **19**: 258–266.

- Saran, R.K., Millar, J.G. and Rust, M.K. 2007. Role of (3Z,6Z,8E)-dodecatrien-1-ol in trail following, feeding, and mating behavior of *Reticulitermes hesperus*. *Journal of Chemical Ecology*, **33**: 369–389.
- Sarmadi, B.H. and Ismail, A. 2010. Antioxidative peptides from food proteins: A review. *Peptides*, **31**: 1949–1956.
- Schock, T.B., Stancyk, D.A., Thibodeaux, L., Burnett, K.G., Burnett, L.E., Boroujerdi, A.F.B. and Bearden, D.W. 2010. Metabolomic analysis of Atlantic blue crab, *Callinectes sapidus*, hemolymph following oxidative stress. *Metabolomics*, **6**: 250–262.
- Shirley, S.M., Shirley, T.C. and Meyers, T.R. 1986. Hemolymph responses of Alaskan king crabs to rhizocephalan parasitism. *Canadian Journal of Zoology*, **64**: 177–1781.
- Shukalyuk, A.I. and Isaeva, V.V. 2000. Intravital and histological studies of the internal organization of the rhizocephalan barnacle *Sacculina polygenea*. *Russian Journal of Marine Biology*, **26**: 209–211.
- Shukalyuk, A.I., Isaeva, V.V., Pushchin, I.I. and Dolganov, S.M. 2005. Effects of the *Briarosaccus callosus* infestation on the commercial golden king crab *Lithodes aequispina*. *The Journal of Parasitology*, **91**: 1502–1504.
- Sillam-Dussès, D., Hanus, R., Abd El-Latif, A.O., Jiroš, P., Krasulová, J., Kalinová, B., Valterová, I. and Šobotník, J. 2011. Sex pheromone and trail pheromone of the sand termite *Psammotermes hybostoma*. *Journal of Chemical Ecology*, **37**: 179–188.
- Silva, A.T., Ligterink, W. and Hilhorst, H.W.M. 2017. Metabolite profiling and associated gene expression reveal two metabolic shifts during the seed-to-seedling transition in *Arabidopsis thaliana*. *Plant Molecular Biology*, **95**: 481–496.

- Sloan, L.M. and Hardy, S.M. 2017. Larval biology and environmental tolerances of the king crab parasite *Briarosaccus regalis*. *The Journal of Parasitology*, **103**: 22–31.
- Sloan, N.A. 1984. Incidence and effects of parasitism by the rhizocephalan barnacle *Briarosaccus callosus* in the Golden King Crab *Lithodes aequispina*, from deep fjords in Northern British Columbia Canada. *Journal of Experimental Marine Biology and Ecology*, **84**: 111–131.
- Sloan, N.A. 1985. Life history characteristics of fjord-dwelling golden king crabs *Lithodes aequispina*. *Marine Ecology Progress Series*, **22**: 219–228.
- Sparks, A.K. and Morado, J.F. 1986. Histopathology and host response in lithodid crabs parasitized by *Briarosaccus callosus*. *Diseases of Aquatic Organisms*, **2**: 31–38.
- Stevens, B.G. 2014. *King Crabs of the World: Biology and Fisheries Management*. CRC Press, Boca Raton.
- Stevens, B.G. and Lovrich, G.A. 2014. Chapter 1: King crabs of the world: species and distributions. In: *King Crabs of the World, Biology and Fisheries Management* (B. G. Stevens, ed), pp. 1–30. CRC Press, Boca Raton.
- Sumner, L.W., Amberg, A., Barrett, D., Beale, M.H., Beger, R., Daykin, C.A., Fan, T.W.-M., Fiehn, O., Goodacre, R., Griffin, J.L., Hankemeier, T., Hardy, N., Harnly, J., Higashi, R., Kopka, J., Lane, A.N., Lindon, J.C., Marriott, P., Nicholls, A.W., Reily, M.D., Thaden, J.J. and Viant, M.R. 2007. Proposed minimum reporting standards for chemical analysis. *Metabolomics*, **3**: 211–221.
- Swetha, C., Sainath, S., Reddy, P. and Reddy, P. 2011. Reproductive endocrinology of female crustaceans: Perspective and prospective. *Journal of Marine Science Research and Development*, **S3**: 1–13.

- Wei, J.J., Zhang, F., Tian, W.J., Kong, Y.Q., Li, Q., Yu, N., Du, Z.Y., Wu, Q.Q., Qin, J.G. and Chen, L.Q. 2016. Effects of dietary folic acid on growth, antioxidant capacity, non-specific immune response and disease resistance of juvenile Chinese mitten crab *Eriocheir sinensis* (Milne-Edwards, 1853). *Aquaculture Nutrition*, **22**: 567–574.
- Wheatly, M.G. 1985. Free amino acid and inorganic ion regulation in the whole muscle and hemolymph of the blue crab *Callinectes sapidus* Rathbun in relation to the molting cycle. *Journal of Crustacean Biology*, **5**: 223–233.
- Xia, J., Psychogios, N., Young, N., Wishart, D.S. 2009. MetaboAnalyst: A web server for metabolomic data analysis and interpretation. *Nucleic Acids Research*, **37**: W652—W660.
- Xia, J., Sinelnikov, I. V., Han, B. and Wishart, D.S. 2015. MetaboAnalyst 3.0-making metabolomics more meaningful. *Nucleic Acids Research*, **43**: W251–W257.
- Yang, J., Zhao, X., Lu, X., Lin, X. and Xu, G. 2015. A data preprocessing strategy for metabolomics to reduce the mask effect in data analysis. *Frontiers in Molecular Biosciences*, **2**.

CHAPTER 4: AUTUMN DISTRIBUTION OF BRISTOL BAY RED KING CRAB USING FISHERY LOGBOOKS³

Abstract

Spatial distributions of fished species must be well characterized to avoid local depletions, identify critical habitat, and predict and mitigate interactions with other fisheries. The Bristol Bay red king crab (*Paralithodes camtschaticus*) fishery is one of the largest crab fisheries in Alaska. Summer crab distributions have been well documented by decades of bottom trawl surveys. However, crab movement and distribution are poorly understood outside the summer survey period, which creates several management challenges. One important component of fishery management is the existence of no-trawl zones, which are intended to protect crab from bottom trawl fisheries. However, it is difficult to evaluate the placement of no-trawl zones, because most crab bycatch occurs in trawl fisheries during winter when crab distributions are unknown. Daily fishing logs, kept by skippers in the red king crab fleet since 2005, contain detailed information on the spatial distribution of catch and effort of legal sized male crab during the autumn crab fishery. However, data contained in these hand-written logbooks have not been readily accessible. We digitized daily fishing logs from 2005 to 2016 and used spatial information on catch and effort to infer geographic distributions of legal sized male king crab during the crab fishing season. Changes in distribution were tracked across this 12-yr period and comparisons were made between warm and cold temperature regimes. In warm years (2005, 2014 – 2016), crab aggregated in the center of Bristol Bay, Alaska, while in cold years (2007 – 2013) they were closer to the Alaska Peninsula. The majority of crab were caught

³ Sloan, L.M., G.H. Kruse, and S.M. Hardy. Autumn distribution of Bristol Bay red king crab using fishery logbooks. In review with PLOS ONE.

in no-trawl areas (63.4% on average), but variations occurred among years and with temperature regime (40.0 – 86.8% in no-trawl zones). As temperatures continue to shift in the Bering Sea, it will be important to continue monitoring crab distributions outside the summer survey period.

Introduction

The exploitation of red king crab (RKC), *Paralithodes camtschaticus*, in Bristol Bay, Alaska, has had a long history, beginning with Japanese harvests in the 1920s [1]. In the late 1960s the domestic fishery greatly expanded, with harvests peaking in 1980 and then rapidly declining over the next few years, resulting in fishery closure in 1983 and again in 1994 and 1995 [2,3]. Since then, Bristol Bay RKC have recovered to smaller yet sustainable stock levels [2] that continue to support one of the most valuable shellfish fisheries in Alaska, with an ex-vessel value ranging over US\$62 - 117 million annually between 2005 and 2014 [4].

The Bristol Bay RKC fishery primarily occurs on the middle shelf of the southeastern Bering Sea, south of 58°N and east of 165°W at depths between 50 and 100 m. This crab stock is co-managed by the Alaska Department of Fish and Game (ADF&G) and the National Marine Fisheries Service (NMFS), but ADF&G is responsible for day-to-day management under the guidance of the federal king and Tanner crab fishery management plan. Historically, this fishery was managed as “limited entry”, with a guideline harvest level (aka catch quota) set each season for those with permits to participate. The large incentive for vessels to “race for fish” and harvest crab faster than competitors, created many environmental, economic, and safety concerns [5]. In 2005, management changed to a crab rationalization program [6], whereby each harvester is now allocated a percentage (individual fishing quota) of the total allowable catch, and there is no longer a need to “race for fish” because quotas can be harvested any time over the three-month season (October 15 – January 15) [7]. This unique program also allocated processing

quota shares, as well as community protections that require a certain portion of the catch to be landed in particular regions [5].

In addition to catch restrictions for the target fishery, Bristol Bay RKC are protected from bycatch in the trawl fisheries through trawl closure areas (Fig. 4.1) and bycatch limits [8]. The Red King Crab Savings Area (RKCSA) was designed to protect adult RKC and prohibits non-pelagic trawls year-round, except in the Red King Crab Savings Sub-Area (RKCSS). Fishing may occur in the sub-area as long as crab abundance in the previous year was high enough to support a directed crab fishery. For the remainder of the manuscript, references to the RKCSA

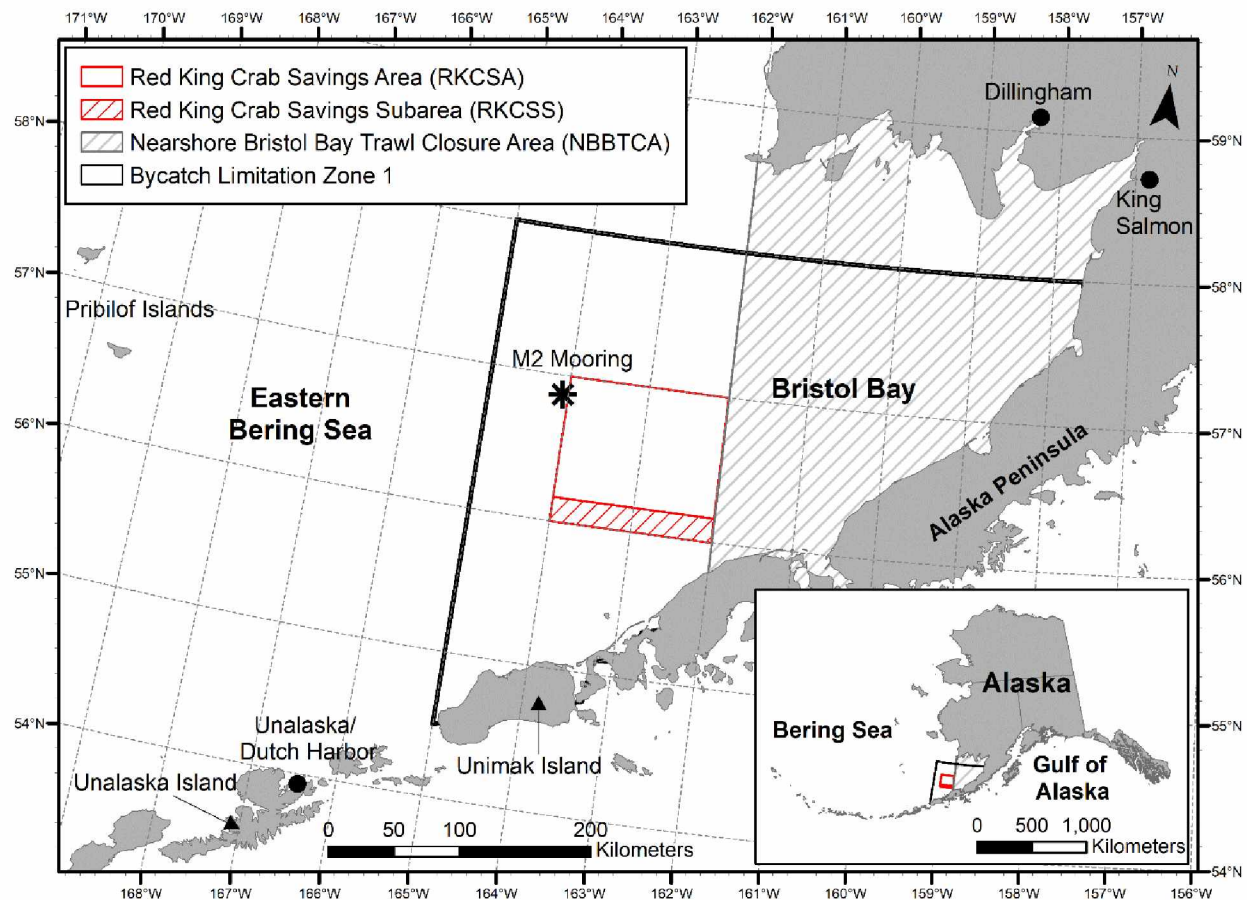


Figure 4.1. Protected areas for Bristol Bay red king crab in the southeastern Bering Sea. Map shows areas closed to bottom trawling (RKCSA and NBBTCA) and open to trawling, but with bycatch limitations (Zone 1 and RKCSS). Asterisk indicates location of M2 Mooring.

pertain only to the non-trawlable portion, excluding the RKCSS. To protect juvenile RKC, non-pelagic trawling is also prohibited in the Nearshore Bristol Bay Trawl Closure Area (NBBTCA). RKC are a prohibited species in non-pelagic trawl fisheries, meaning that when a designated bycatch limit is reached, all of Zone 1 (Fig. 4.1) is closed to non-pelagic trawling [8].

RKC distributions vary over both seasonal and interannual time scales due to ontogeny, seasonal reproductive cycles, and variable environmental factors. RKC migrate to shallow waters in late winter, and larval release, molting, and mating occur in the spring [9–11]. In the Bering Sea, female RKC typically remain in shallow waters, while males migrate to deeper waters in the late summer and autumn [12]. However, decadal-scale trends in temperature also lead to shifts in distribution of benthic species, including RKC [3,13]. The Bering Sea oscillates between warm and cold temperature regimes, largely driven by sea ice extent [14,15]. In cold years, with greater sea ice extent and later ice retreat in the spring, a pool of cold ($< 2^{\circ}\text{C}$) bottom water (called the “cold pool”) persists in the southeastern Bering Sea throughout the summer and into autumn until vertical mixing occurs [14]. In contrast, the cold pool is further north in warm years [13], and bottom waters in the southeastern Bering Sea are several degrees warmer [14]. Over the past 12 years, 2005 and 2014 – 2016 were warm years, 2006 was a moderate year, and 2007 – 2013 were cold years [15]. In recent cold years (e.g., 2008, 2009, 2010, 2012), summer distribution of RKC shifted from central Bristol Bay to nearshore regions along the Alaska Peninsula [16]. Shifts in distribution, particularly if unaccounted for in management efforts, are cause for concern, because they may leave crab more vulnerable to habitat disturbance and unintended bycatch [16].

Stock assessments for Bristol Bay RKC are primarily based on the NMFS eastern Bering Sea continental shelf bottom trawl survey for crab and groundfish [17]. This survey has been

conducted annually since 1975 in early June through late July/early August, providing an excellent time series with which to examine abundance trends and summer crab distributions. However, no complimentary survey is conducted during the autumn or winter. Thus, our knowledge of autumn/winter crab distributions is based on catch data from the fishing industry collected from fish tickets (landing records), and by onboard observers and port samplers. These data are generally reported by large ADF&G statistical areas (0.5° latitude x 1° of longitude) [18], that do not allow for examination of fishing effort or crab distributions on a finer spatial scale. Data on the spatial distribution of crab bycatch in groundfish fisheries are also available, but only for areas where trawling is permitted. Lack of detailed data on winter RKC distributions is of concern because most RKC bycatch in non-pelagic groundfish trawls occurs in the winter, especially in the rock sole (*Lepidopsetta* spp.) fishery [16,19]. Recognizing the effects of temperature on crab distribution, the North Pacific Fishery Management Council called for an examination of the effectiveness of the current trawl closure areas in Bristol Bay in reducing RKC bycatch [16,20]; however, without detailed data on winter RKC distributions, the effectiveness of trawl closure areas has been difficult to evaluate. This study used catch per unit effort (CPUE) data from fishermen's logbooks during the autumn RKC fishery to infer crab distributions shortly before the start of winter trawl fisheries.

Daily fishing logs (DFLs), kept by skippers in the RKC fleet since rationalization in 2005, can help improve our understanding of legal sized (≥ 165 mm carapace width) male RKC distributions outside the summer survey period. DFLs contain detailed information on RKC catch and location during the late autumn/early winter fishery, especially for the first month of the fishery when the majority of crab quotas (74% – 100%) are filled (October 15th – November 14th). DFLs are hand-written by skippers, and carbon copies are submitted to NMFS and

ADF&G each year. In addition to DFLs, onboard observer data also provide information on RKC distributions within each fishing season. Since 2005 Bristol Bay RKC observers have recorded detailed data on the catch in sampled pots (aka traps), but they only sample approximately 5% of the pots on 20% of the vessels, equating to ~1-2% of total pots in the fishery annually [18]. Rather than very specific information on just a few pots, DFLs provide an average catch for each pot string (group of ~30 pots) across the entire fishery.

We digitized DFLs from 2005 through 2016 to elucidate the spatial and temporal changes in the winter distribution of legal Bristol Bay RKC. We compared patterns from DFL data to information gained from observer data to determine if these two different sources of information collected from the same fishery yielded similar spatial patterns. Although observer data are more precise for the pots sampled, there are fewer observations, and thus they may not cover the same spatial area. Based on autumn crab distributions from DFLs, we evaluated the effectiveness of the trawl closure areas, and how shifts in RKC distribution relate to large-scale temperature regimes in the Bering Sea.

Methods and Approach

Daily fishing log data preparation

DFLs are hand written on carbon paper, with 5 copies of each entry; ADF&G and NMFS each get one copy, while the original stays with the vessel. We used ADF&G copies because archived logs were more accessible and ADF&G copies were expected to be more legible, as they are the second carbon copy, whereas NMFS has the fourth carbon copy. Data from all Bristol Bay RKC DFLs from 2005 through 2016 were entered by hand into spreadsheets, accounting for 29,973 records. Very few records (< 1%) were illegible or incomplete, with an unknown number of DFL pages missing. To evaluate the completeness of these records we

compared total catch for each year from DFL records to the fishery records of total catch recorded in the crab stock assessment and fishery evaluation (SAFE) reports [21]. For 2016, we obtained the total catch information directly from ADF&G (Dutch Harbor, AK office), since it was not yet recorded in a SAFE report. Figure 4.2 shows that these DFL records encompass a large proportion of the total crab fished each year, from 87.5% in 2005 to 96.6% in 2008.

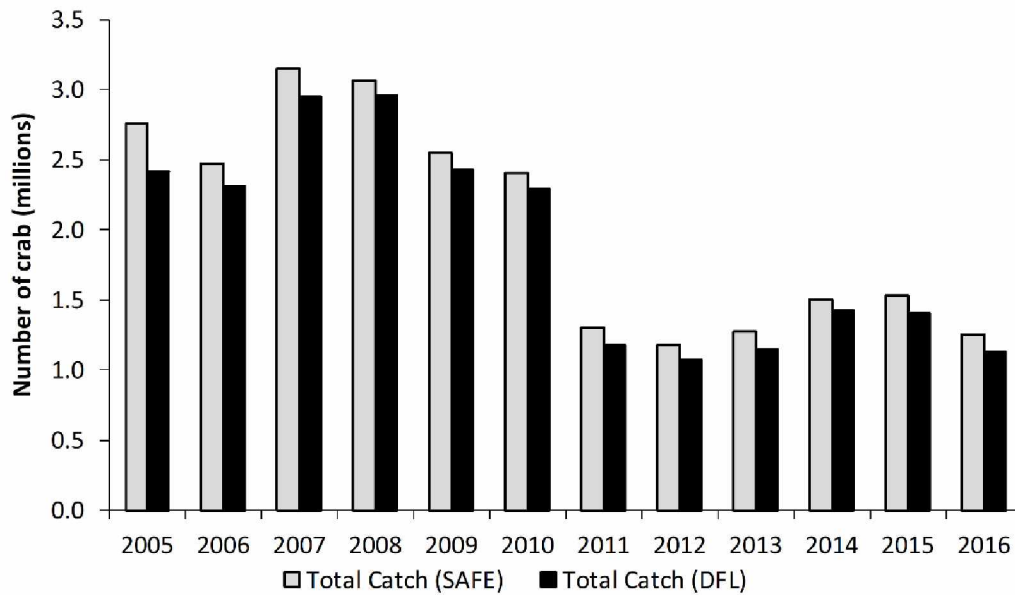


Figure 4.2. Bristol Bay red king crab catch over 2005 – 2016. A comparison of catch reported in daily fishing logs (DFLs) and in the crab stock assessment and fishery evaluation (SAFE) report ($R^2 = 0.993$).

In the Bristol Bay RKC fishery, crab pots are generally set in straight rows, called strings (29.5 ± 23.9 pots [mean pots per string ± 1 SD]; 10.4 ± 10.2 km [mean string length ± 1 SD]). Each DFL entry represents an entire string, not an individual pot, with the following recorded: coordinates and depth of the first and last pot in each string, date and time pots were set and retrieved, number of pots set and lost, and number and weight of legal RKC caught and retained. Catch per pot was calculated for each DFL string as the number of crab caught divided by the number of pots hauled (pots set minus pots lost). Soak time (63.6 ± 47.3 hours [mean ± 1 SD])

had little effect on catch per pot ($R^2 = 0.05$), thus it was not taken into account in these analyses, and nominal catch per pot is used for CPUE.

To eliminate biases from extreme values, data were trimmed in two ways for spatial analyses. First, only strings with > 5 and ≤ 100 pots were included. Strings with only a few pots have a small sample size and are likely to give highly variable values for CPUE, while strings with many pots are unlikely to be set in straight lines and likely cover a larger spatial area than can be understood by the two sets of coordinates provided. In addition, strings with a linear length greater than 20 km were excluded, because they did not provide sufficiently fine spatial resolution. After trimming, records for 26,892 strings remained (90% of total data). The resultant mean string length was reduced to 8.0 ± 3.6 km (± 1 SD), and the number of pots per string was reduced to 25.4 ± 12.0 (± 1 SD). For each trimmed record, the string midpoint was calculated by averaging the start and end coordinates of the string. The string midpoints were used in all subsequent spatial analyses.

Spatial analysis

In all spatial analyses, the spatial relationship, or spatial neighborhood, must be defined around each observed value (i) of the variable X of interest (i.e., CPUE) [22,23]. A matrix of spatial weights (w_{ij}) explains how each value of i relates to all other observed values (j), and which values (j) are included in the neighborhood around each value (i) [22]. Spatial weights define the scale of the analysis, and they affect the patterns in autocorrelation across the study area [22,23]. Neighborhood weights can be defined in a variety of ways, including distance (d) from i or the k closest points to i [22]. Distances can be fixed, or they can decay with distance from i [24]. We tested five different neighborhood weighting methods, including a fixed $d = 5$, 10, and 20 km and $k = 20$ and 40 points (Fig. 4.1 Sup.). A k of 20 and 40 gave similar results to

a d of 5 and 10, respectively. However, using a fixed number of neighbors resulted in inherent biases with our data. Data points tended to be sparser in low CPUE areas that fishermen actively avoided, and very dense in high CPUE areas that fishermen targeted. Thus, when k was used to define a neighborhood, the low CPUE regions incorporated a greater area around each data point compared with the high CPUE regions. These differences in scale with CPUE made interpretation of results difficult, and we elected to use fixed distance bands, as they provide a uniform scale. Given that point data came from the midpoints of pot strings, and strings were restricted to a maximum of 20 km, the longer strings (>10 km) would not fit within the 5 km distance band. The 20 km distance band was so large that it only gave broad patterns, especially in years when fishing occurred over a relatively small area. A distance band of 10 km gave a finer scale view of spatial relationships, while allowing the entire length of all strings to fall within the 10 km distance band of their midpoints. Therefore, results are shown using the 10 km distance band, as it is the most reasonable for this dataset; sample results with 5 km and 20 km distances are provided in the supplemental material (Fig. 4.1 Sup.). Given our choice for neighborhood weights, the matrix w_{ij} is a matrix of zeros and ones. When j is within 10 km of i , the weight is 1, meaning that j is included in the neighborhood of i .

Moran's I , Getis-Ord G_i^* , and local Moran's I statistics were used to identify patterns in the spatial structure of CPUE in string midpoints from the DFL dataset [25–27]. Moran's I is a global statistic used to test for spatial autocorrelation in CPUE across the entire study area [26]:

$$I = \frac{n}{\sum_{i=1}^n \sum_{j=1}^n w_{i,j}} \frac{\sum_{i=1}^n \sum_{j=1}^n w_{i,j} (x_i - \bar{X})(x_j - \bar{X})}{\sum_{i=1}^n (x_i - \bar{X})^2}, \quad (4.1)$$

where \bar{X} is the global mean CPUE, and x_i and x_j are the CPUEs at value i and j , respectively. A Moran's $I > 0$ indicates clustering of similar values, $I < 0$ indicates clustering of dissimilar values, and $I = 0$ indicates no autocorrelation or perfect randomness.

The Getis-Ord G_i^* statistic was used to measure local spatial clustering and identify areas of high (hot spots) and low (cold spots) CPUE [25]:

$$G_i^* = \frac{\sum_{j=1}^n w_{i,j} x_j - \bar{X} \sum_{j=1}^n w_{i,j}}{\sqrt{\frac{\sum_{j=1}^n x_j^2}{n} - \bar{X}^2} \sqrt{\frac{n \sum_{j=1}^n w_{i,j}^2 - (\sum_{j=1}^n w_{i,j})^2}{n-1}}}. \quad (4.2)$$

The G_i^* statistic measures positive spatial autocorrelation. The calculation is performed for every data point (string midpoint), i , which tests the null hypothesis that the mean of i and its neighbors is equal to the global mean. When the null hypothesis is rejected, indicated by a significant p -value, i is a hot spot when G_i^* is positive and a cold spot when G_i^* is negative. Due to the nature of this dataset, cold spots are generally data-poor, since fishermen try to avoid these locations. Although the locations of cold spots are shown, we focus our analyses and interpretation on hot spots.

Local Moran's I was used as a secondary local statistic to verify results from the G_i^* statistic. This statistic is the local equivalent of the global Moran's I , with:

$$I_i = \frac{x_i - \bar{X}}{\frac{\sum_{j=1, j \neq i}^n w_{i,j} - \bar{X}^2}{n-1}} \sum_{j=1, j \neq i}^n w_{i,j} (x_j - \bar{X}). \quad (4.3)$$

Moran's I_i measures both positive and negative spatial autocorrelation, thus four significant situations can arise: high-high (i is higher than the mean and so are its neighbors), low-low (i is lower than the mean and so are its neighbors), high-low (i is high, but its neighbors are low), and low-high (i is low, but its neighbors are high). Only the results from the high-high scenario are shown in results for comparison with the G_i^* hot spots.

When local autocorrelation analyses are performed in the presence of global autocorrelation, the probability of a Type I error increases, because data points may not be independent, and multiple comparisons are performed [27,28]. However, corrections for multiple comparisons, like Bonferroni, are too conservative, because they assume that all data

points are dependent, which is unlikely [23,27]. To be cautious, we defined significance at $\alpha = 0.01$ and all p-values were calculated using permutations [29]. Observed statistics were compared with reference distributions created from 10,000 permutations of spatially random CPUE data. Pseudo p -values were calculated using one-sided significance tests, such that $p = (M + 1)/(R + 1)$. Here, R is the number of permutations and M is the number of times the permuted statistic is greater than or equal to the observed statistic (for positive G_i^* and I_i) or less than or equal to the observed statistic (for negative G_i^* and I_i) [30].

Mapping

For each year, two maps were created showing the distribution of G_i^* and I_i statistics, first using CPUE data for the entire season, and second using only data from the beginning of the fishery. We defined the “beginning” of the fishery as the first 5% of crab caught out of the total legal crab in the population (hereafter referred to as “first 5%”). Total legal crab abundances in the Bristol Bay RKC population are estimated in the SAFE reports each year [21]. Continued fishing effort drives down the global mean CPUE, and the total allowable catch varies among years, so hot spot analyses are not directly comparable among years when all data are used. The fishing location and catch information of crab vessels is confidential. Therefore, the string midpoint data (all data points, i) used in analyses cannot be shown here. To show allowable approximations of these confidential data, we aggregated data points into large irregular polygons. All aggregations were performed using a 10-km aggregation distance, meaning that data points within 10-km of each other were aggregated into the same irregular polygon. Three aggregations were performed for each map. First, all data points, i , were aggregated to visualize total fishing extent for each year (i.e., combination of red, blue, yellow areas in Fig. 4.3). Second, all data points, i , that were determined to be hot spots were aggregated (i.e., red areas in

Fig. 4.3). Third, all data points, i , that were determined to be cold spots were aggregated (i.e., blue areas in Fig. 4.3).

A third map was made for each year showing the distribution of total catch, rather than significant areas of high CPUE. A hexagonal grid with 100 km² grid cells was placed over the study area, and the total number of crab was summed in each cell. To adhere to confidentiality requirements, only grid cells containing data from 3 or more vessels are shown.

Hot spots were extracted from each year and all years were overlaid on each other to examine hot spot persistence across years. The same was done for high catch regions ($\geq 20,000$ crab/grid cell). Because warm and cold years showed different RKC distributions, they were mapped separately. All spatial analyses were conducted in GeoDa version 1.8 [29], while data cleaning, pre-processing, and mapping were done in ArcGIS version 10.3 [31]. Data were projected onto the Alaska Albers Equal Area Conic using the North American 1983 Datum.

Observer data

Observers report the catch and latitude/longitude coordinates of individual pots, not strings, thus it was not necessary to trim observer data and the entire dataset was used for each year. Across the 12 focal years, observers recorded information on 13,813 pots. Observer data were analyzed using the Moran's I and G_i^* statistics and mapped by aggregating points, as described above. Given that the observer dataset has fewer observations than the DFL dataset, cold spots were poorly resolved, thus we only show hot spots here.

Results

On the global scale, positive spatial autocorrelation occurred in all years, when using observer data ($I = 0.046 - 0.259$; $p < 0.001$) and DFL data from the entire fishing season ($I = 0.090 - 0.295$; $p < 0.001$) and only the first 5% ($I = 0.124 - 0.563$; $p < 0.001$). On the local

scale, statistically significant hot spots and cold spots occurred in all years, in all datasets (Figs. 4.3 and 4.4).

Hot spot validation

Hot spots detected using G_i^* and I_i show virtually identical results (Fig. 4.3). A few small hot spots were only detected in one of these analyses (e.g., 2006); henceforth, we only consider hot spots detected using both statistics. Hot spots detected using observer data generally overlapped with those identified using DFL data, although the degree of correspondence varied among years (Fig. 4.3). In many instances, the locations of DFL and observer hot spots were very similar (e.g., 2005, 2008, 2012), while in other years they did not overlap as much, or some hot spots were only detected in one of the datasets (e.g., 2010, 2011). However, observer hot spots were always near DFL hot spots and never overlapped DFL cold spots.

In general, hot spot maps from the first 5% corresponded well with the entire dataset, with most hot spots being represented in both maps (Figs. 4.3 and 4.4; black numbered hot spots). However, some hot spots only appeared in one of the maps (Figs. 4.3 and 4.4; red numbered hot spots). Both maps had the same hot spots in 2005 and 2012, with all other years having at least one difference. For the most part, extra hot spots occurred in the full dataset that did not occur in the first 5%. In some instances, fishing had not yet occurred in these locations in the first 5% dataset (e.g., 2006 hot spot 2, 2007 hot spot 2, 2009 hot spot 3, and 2015 hot spot 2), but in other cases fishing had occurred over at least part of the area, but the area was not considered a hot spot (e.g., 2008 hot spot 1, 2011 hot spot 4, 2016 hot spot 1). In a few years, hot spots occurred in the first 5% dataset, but not in the full dataset (e.g., 2006 hot spot 3, 2009 hot spot 5, 2014 hot spot 5).

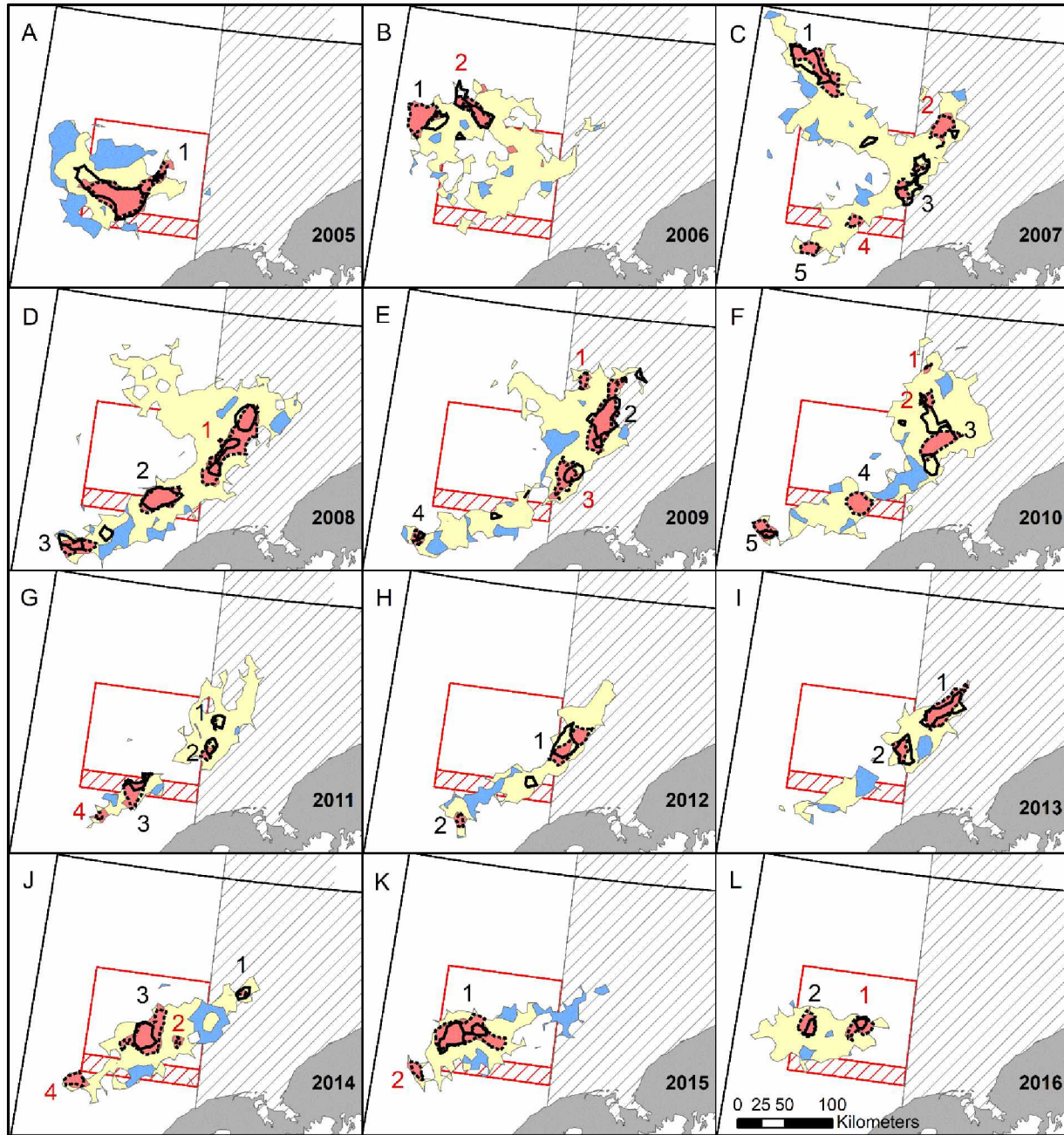


Figure 4.3. Red king crab distributions using daily fishing logs (DFLs) from the entire season. Red, blue, and yellow areas indicate locations where fishing occurred each fishing season (October 15th – January 15th). Red areas are detectable hot spots (G_i^* indicating statistically significant ($\alpha < 0.01$) high catch per unit effort (CPUE)); blue areas are detectable cold spots (G_i^* indicating statistically significant low CPUE); yellow areas indicate locations where CPUE was not statistically different from the mean. Black dashed lines are hot spots using the local Moran's I statistic on DFL data and solid black lines are hot spots using observer data (G_i^* statistic). Hot spots are numbered in color for each year, black when they occur in both the full dataset (this figure) and the first 5% (Fig. 4.4) and red when they occur in just one dataset. Areas with restrictions on trawling are outlined in red or gray and are described in Figure 4.1.

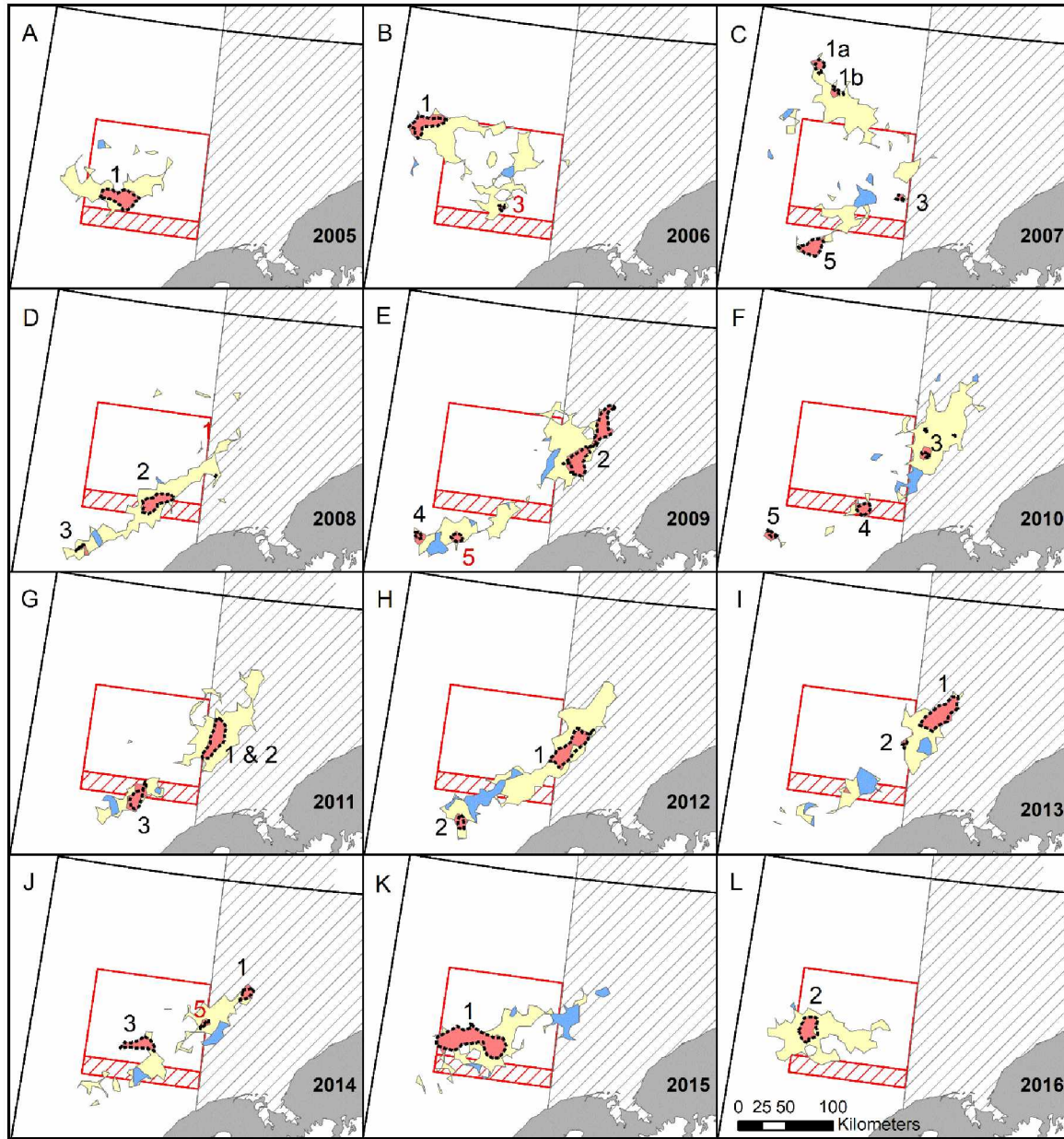


Figure 4.4. Red king crab distributions using daily fishing logs (DFLs) from the beginning of the season. The beginning of the fishing season (entire season October 15th – January 15th) was defined as the first 5% of crab caught out of total legal crab in the population. Red, blue, and yellow areas indicate locations where fishing occurred. Red areas are detectable hot spots (G_i^* indicating statistically significant ($\alpha < 0.01$) high catch per unit effort (CPUE)); blue areas are detectable cold spots (G_i^* indicating statistically significant low CPUE); yellow areas indicate locations where CPUE was not statistically different from the mean. Black dashed lines are hot spots using the local Moran's I statistic on DFL data and solid black lines are hot spots using observer data (G_i^* statistic). Hot spots are numbered in color for each year, black when they occur in both the full dataset (Fig. 4.3) and the first 5% (this figure) and red when they occur in just one dataset. Areas with restrictions on trawling are outlined in red or gray and described in Figure 4.1.

Hot spots versus high catch areas

Overall, the patterns in total catch per grid cell (Fig. 4.5) gave similar results to the hot spot analyses (Fig. 4.3). For some years, the hot spots and high catch areas ($\geq 20,000$ crab/ 100 km²) were almost identical (2010 and 2015), but for most years some differences occurred. High catch areas generally fell over hot spot areas, but they often also occurred in areas without hot spots. For example, there are high catch areas in the RKCSS in 2012 and 2013, but this area is not a hot spot in either year. Occasionally, high catch areas even occurred in the same location as cold spots (e.g., 2014). Figure 4.6 shows hot spots and high catch areas that persisted for at least two years. These two metrics give very similar results, except for the area just south of the RKCSA, which is consistently a high catch area, but not a hot spot.

Distribution with temperature regime

In warm and cold years, hot spots occurred in different locations in Bristol Bay (Fig. 4.7). In warm years (2005, 2014 – 2016), hot spots consistently fell in central Bristol Bay within the RKCSA (Fig. 4.7A). In contrast, in cold years (2007 – 2013), hot spots occurred in a band along the Alaska Peninsula and further east in Bristol Bay (Fig. 4.7B). The exact locations and intensities of hot spots varied among cold years, but in general they were 1) south of the western section of the RKCSS, 2) in the eastern portion of the RKCSS, and 3) east of the RKCSA in the NBBTCA. In 2006, which was a moderate transition year between previous warm and subsequent cold years, the distribution was different, with a hot spot to the northeast of the RKCSA (Fig. 4.3). This northern hot spot also occurred in 2007 (Fig. 4.3).

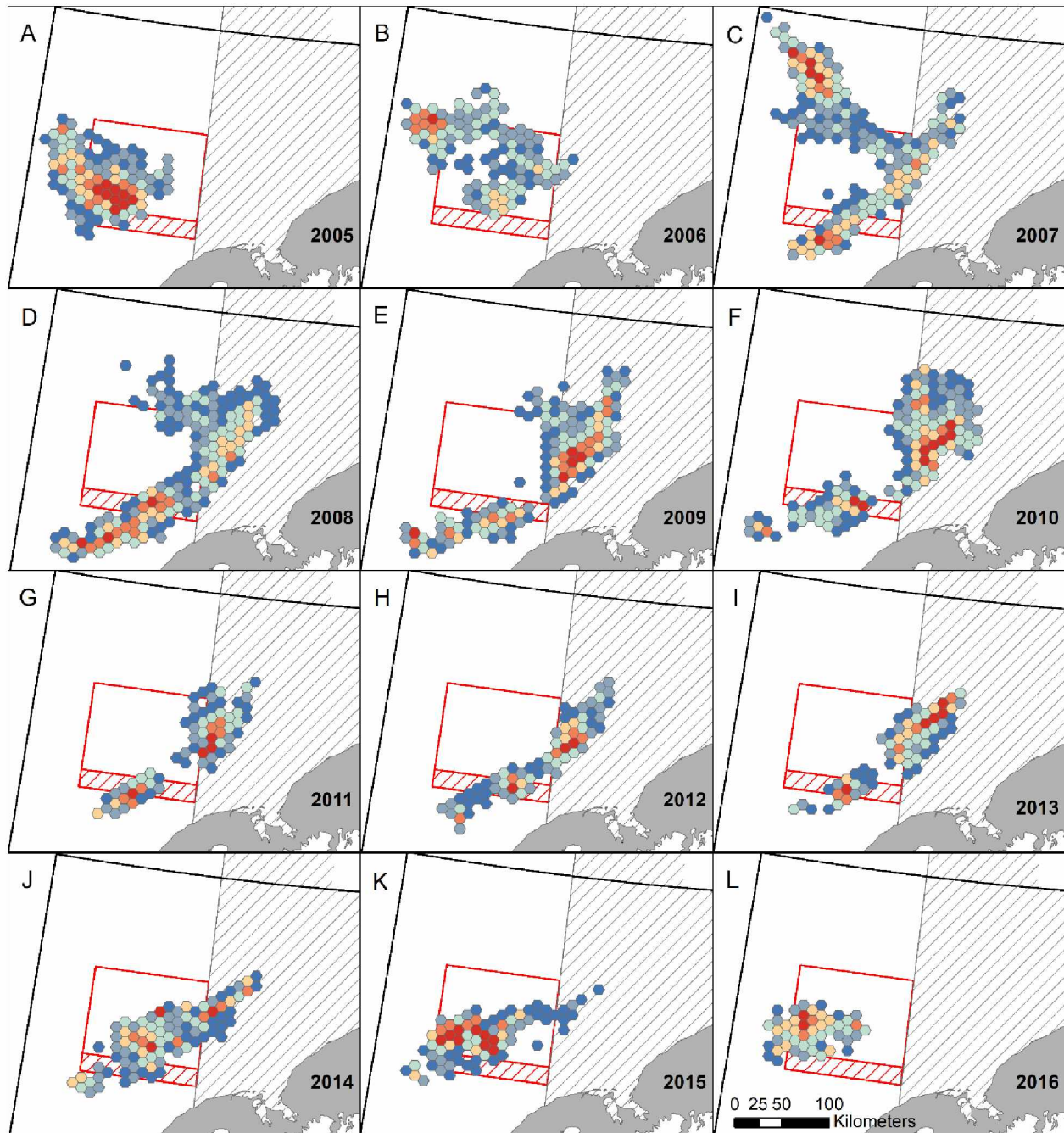


Figure 4.5. Total catch of legal male red king crab in Bristol Bay using daily fishing logs. Hexagonal grid cells (100 km^2) show the number of crab caught each fishing season (dark red $\geq 40,000$ crab, medium red = 30,000 – 39,999, light red = 20,000 – 29,999, light blue = 10,000 – 19,999, medium blue = 5,000 – 9,999, and dark blue $< 5,000$). Only grid cells represented by ≥ 3 vessels are included due to confidentiality restrictions. Areas with restrictions on trawling are outlined in red or gray and are described in Figure 4.1.

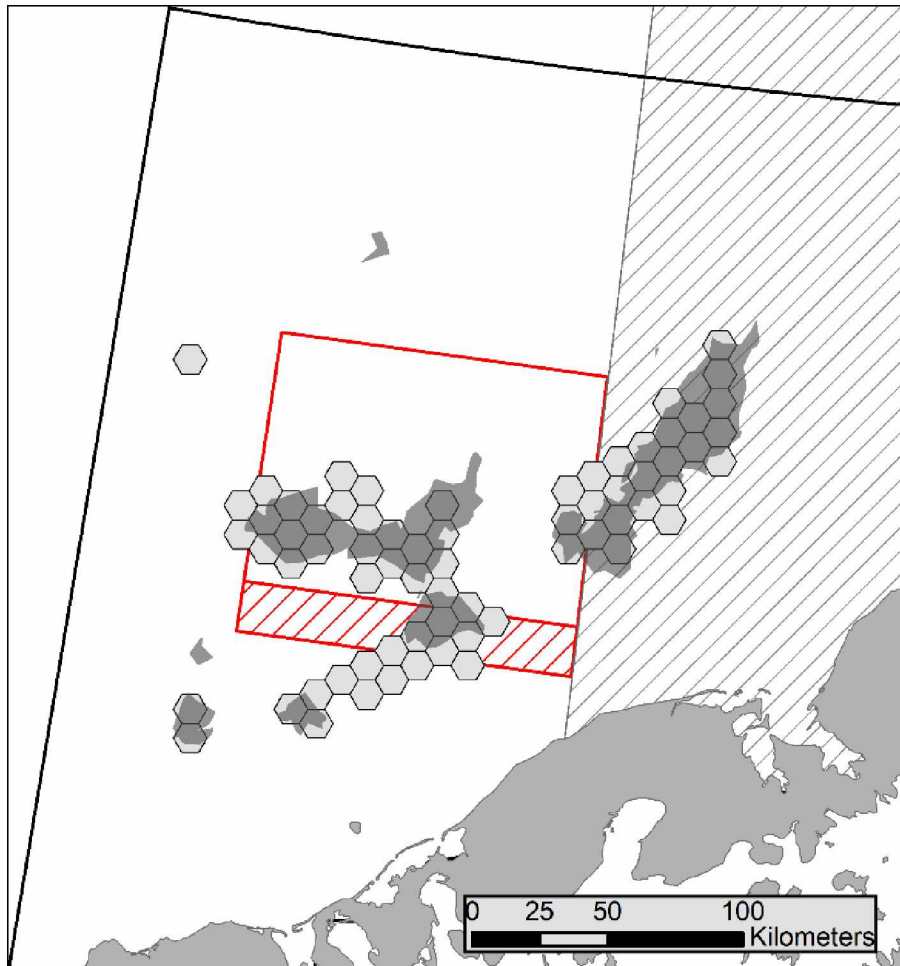


Figure 4.6. Persistent hot spots and high catch areas for red king crab over 2005 – 2016. Irregular polygons are hot spots (Getis-Ord, G_i^* ; Fig. 4.3) that persisted in those areas for at least two years and hexagonal polygons had a high crab catch ($\geq 20,00$ crab caught; Fig 4.5) for at least two years. Areas with restrictions on trawling are outlined in red or gray and described in Figure 4.1.

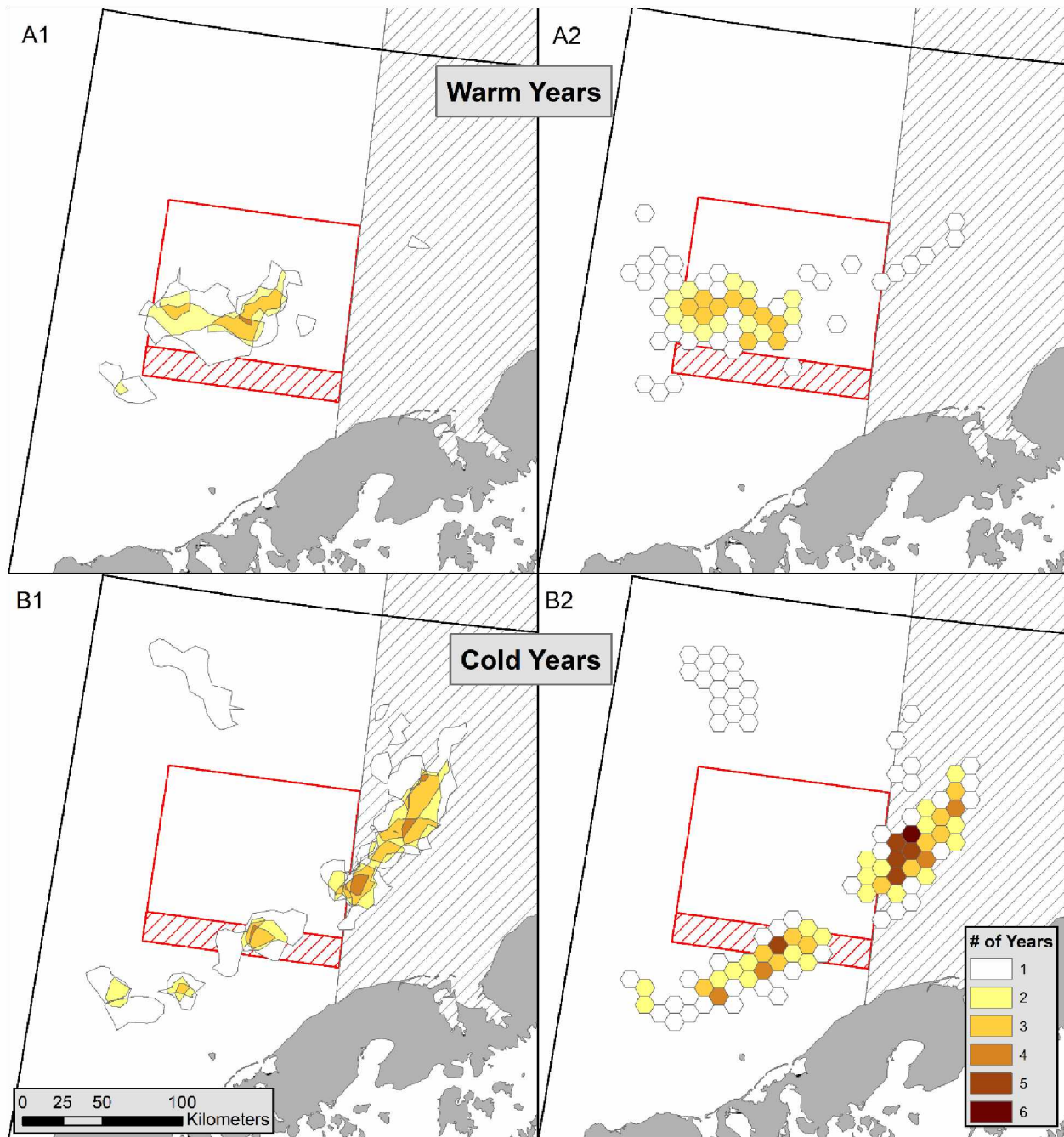


Figure 4.7. Hot spots and high catch areas for red king crab in warm and cold years. Getis-Ord hot spots, G_i^* for catch per unit effort (A1, B1; red areas from Fig. 4.3) and high crab catch areas (A2, B2; red grid cells in Fig. 4.5) for Bristol Bay red king crab using daily fishing logs for A) warm years (2005, 2014 – 2016) and B) cold years (2007 – 2013) in the Bering Sea (characterized after Duffy-Anderson (2017) [15]). The legend indicates the number of years a hot spot or high catch area occurred in that location. Areas with restrictions on trawling are outlined in red or gray and described in Figure 4.1.

Trawl closure areas

Over the 12-year period, 63.4% of the RKC catch occurred in no-trawl zones (Fig. 4.8). On average, 32.2% of crab were caught in the RKCSA, 7.8% in the RKCSS, 31.1% in the NBBTCA, and 28.8% in other areas that can be trawled. In general, more crab were caught in closure areas in warm years than in cold years, with 2006, 2007, and 2008 having the lowest number of crab caught in protected areas.

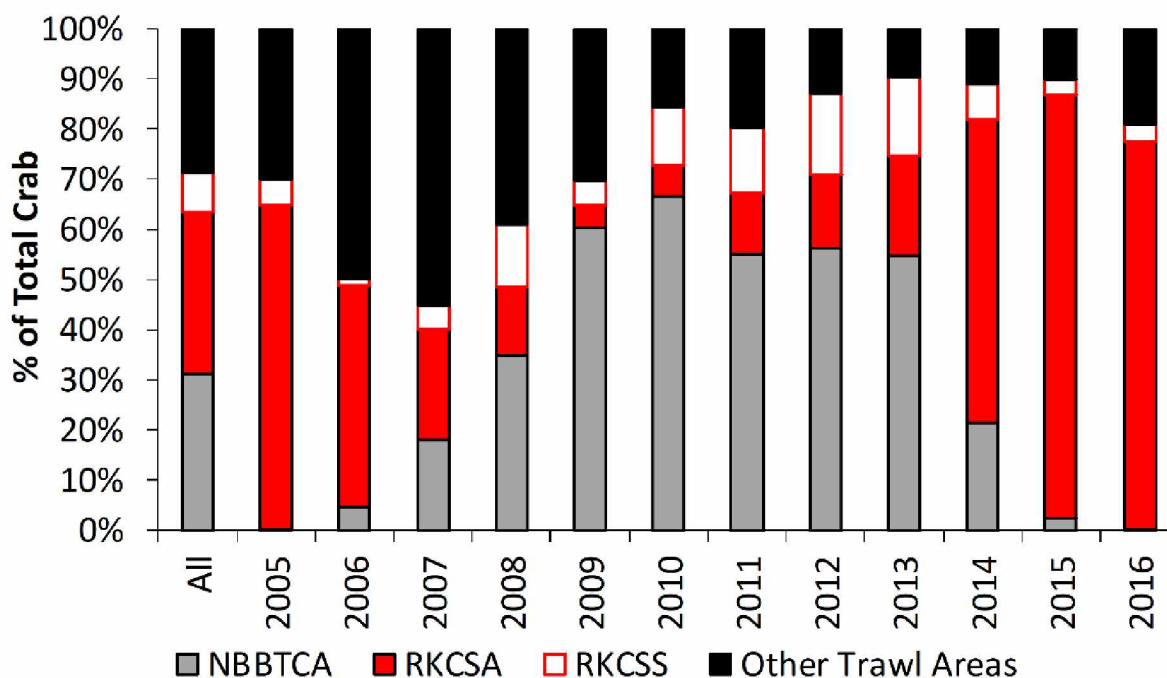


Figure 4.8. Bristol Bay red king crab catch across protected areas for 2005 – 2016. Bars show percent of red king crab caught in trawlable areas (RKCSS, Other) and trawl closure areas (NBBTCA, RKCSA) in the Bristol Bay red king crab fishery. NBBTCA = Nearshore Bristol Bay Trawl Closure Area, RKCSA = Red King Crab Savings Area, RKCSS = Red King Crab savings Sub-Area.

Discussion

Here, we displayed the usefulness of DFLs in revealing the spatial distribution of a highly mobile commercial species across Bristol Bay, during a season when survey data are not available. The high correspondence of spatial patterns in crab distributions between DFLs and observer data helps to validate the accuracy of both datasets. Spatial analyses showed very different patterns in crab distribution depending on temperature regime in the Bering Sea. In warm years, crab were aggregated in a central location within the RKCSA, while in cold years they were concentrated in a band along the Alaska Peninsula. Most crab were harvested within the trawl-closure areas, yet the importance of each closure area varied by year and temperature regime.

Crab distribution

Autumn distribution data from DFLs and observer reports add a new layer to our previous understanding of RKC distributions from the well-established NMFS summer trawl surveys. Comparing RKC distributions inferred from the NMFS survey and DFLs is challenging because of differences in sampling design and area covered (Figure 4.2 Sup.). The NMFS survey has equal sampling effort across most of the continental shelf, with one trawl per 20 x 20 nautical mile grid cell. In contrast, the total area covered by DFL data is more restricted, but the sampling effort within the localized area is higher, although not uniform. Nonetheless, compared with autumn DFL data, NMFS summer data typically show higher numbers of legal male crab further north and east in Bristol Bay and/or closer to the Alaska Peninsula. The differences between summer and autumn distributions of Bristol Bay RKC show the importance of examining distributions at different times of year, especially when these data inform fishery management decisions.

Hot spot analyses (Figs. 4.3 and 4.4) and total catch (Fig. 4.5) provided two distinct metrics for examining autumn distribution of RKC in Bristol Bay. Hot spots are defined here as areas with significantly higher CPUE than the annual mean. In contrast, high catch areas are places where large numbers of crab were extracted over the fishing season ($\geq 20,000$ crab/100 km² grid cell). When a location was a hot spot, but not a high catch area, the CPUE was high, but there was not enough sustained effort in that location to catch at least 20,000 crab/100 km²; it is not possible to determine if these locations would have had enough crab to become high catch areas if fishing effort had been higher. High catch areas that were not hot spots had large numbers of crab extracted, but CPUE was not higher than average. These areas are consistently important areas for crab harvest, but they likely do not have large localized aggregations that can produce high CPUE. In general, hot spots and high catch areas overlapped, verifying the importance of these areas as crab habitat using two separate methods (Fig. 4.6). One important area that was consistently a high catch area, but never a hot spot was the region just south of the RKCSS (Figs. 4.3 and 4.5). These results suggest that crab are abundant in this region, but they are not highly aggregated, causing CPUE to be average, while large numbers crab are extracted.

Temperature effects

Bristol Bay RKC distributions have primarily been studied for female crab around the breeding season in late spring and early summer [3,10,11,32]. Adult females and at least a portion of the adult males migrate to nearshore areas for mating in the spring [10,11,33]. Migrations out of nearshore areas after breeding occur later in colder years [10,11], because cold temperatures delay embryo maturation and mating [34,35]. The extent and location of the Bering Sea cold pool not only affects the timing of these migrations, but is likely responsible for larger inter-annual shifts in spring distribution of female crab [3,10,32,36]. For example, in the

1970s when there was a large cold pool in Bristol Bay, female crab mostly occurred in southwestern Bristol Bay, near Unimak Island (Fig. 4.1). When temperatures warmed in the late 1970s and early 1980s, female crab distributions shifted to central Bristol Bay [3]. Overall, female RKC appear to avoid waters $< 2^{\circ}\text{C}$ to remain in optimal temperatures for embryo development [10].

The influence of temperature on the distribution of male RKC is poorly understood [10]. During the breeding season, at least a portion of the mature male population must occur at the same nearshore sites as females, and thus be governed by the female's temperature requirements. However, in the autumn, male RKC are generally not associated with females [37]. DFL data clearly show that legal sized male crab have distinctly different autumn distributions during warm and cold temperature regimes (Fig. 4.7). The influence of temperature on distribution was particularly apparent in 2014, the first warm year in almost a decade, when male crab returned to the location they had inhabited during the previous warm year, 2005 (Figs. 4.3-4.5). There are few historical data on autumn RKC distributions with which to determine if these patterns hold over longer timescales. At least in some years in the 1960s, the autumn Japanese tangle net grounds were located west of the RKCSA [33,38], where few crab were captured in our study. However, it is unclear if this represents a difference in autumn distribution or timing of the fisheries. For example, in 1967, the so called autumn tangle net fishery occurred from July to September [33], while in our study most crab were caught between October 15th and November 14th. In the United States domestic fishery, ADF&G reports catch by large statistical areas. Comparing a very cold year (1999) and a very warm year (1990) in the Bering Sea, the statistical areas are too coarse to determine whether similar distribution patterns hold as described herein; however, in both of these years more crab were caught in northern quadrants of the RKCSA than

occurred in any years reported here [39,40]. This discrepancy points to the importance of continued monitoring of the autumn distribution of legal male RKC, as future shifts in distribution are possible.

Although shifts in RKC distribution appear to be related to temperature regime, the mechanism behind the male crab response to these temperature shifts outside the breeding season requires further exploration. RKC can tolerate a wide range of temperatures; over 30 years of summer NMFS trawl surveys in the Bering Sea, RKC were found in areas where bottom temperatures were between -0.8 and 12.8 °C, with an average of 3.2 °C [41]. Bering Sea crab may migrate offshore to encounter cooler waters during warm years [42], or may avoid extremely cold water in years when the cold pool is present in Bristol Bay [3]. We propose the former is more probable for legal male crab during the autumn fishery. Regardless of temperature regime, autumn bottom water temperatures are the warmest that crab experience over the course of a year, due to vertical mixing of warm surface waters in autumn [14]. In a laboratory study, when placed in a thermal gradient, mature male RKC occurred across the gradient (< 1 °C to 14 °C), but they typically avoided temperatures > 4 °C and on average favored waters between 2.7 and 3.0 °C [43]. Adult males did not avoid the coldest waters and occurred equally at 0 – 2 °C and 2 – 4 °C water [43]. In years when the cold pool is present in Bristol Bay, water temperatures in the autumn rarely exceed 4 °C at the M2 mooring (Fig. 4.1); however, in warm years bottom temperatures generally exceed 4°C for several months in the autumn [14]. Thus, it is more likely that adult male crab move to avoid warm waters > 4 °C during warm years, rather than avoiding cold waters in cold years. Unfortunately, bottom water temperature is not measured across Bristol Bay in the autumn, so temperature comparisons between areas occupied by crab in warm years and cold years cannot be made.

Regardless of the causes for the observed shift in distribution, there could be consequences for crab living in different habitats. Moving westward, out of Bristol Bay, the sediment becomes finer [44]. In cold years, crab hot spots mostly occur where there is a sandy substrate, while in warm years the substrate in hot spot areas is a mud-sand mixture [44]. Sediment grain size is well known to influence benthic community structure [45,46]. In the southeastern Bering Sea, sediment type can dictate diet and distribution of flatfishes [47]. RKC are generalist predators, feeding on a wide variety of organisms, including molluscs, echinoderms, polychaetes, and cnidarians [48]. The influence of substrate type on the distribution of benthic prey species suggests that changes in crab distribution will cause (or perhaps be driven by) shifts in the composition of their diet. For example, yellowfin sole (*Limanda aspera*) in the southern Bering Sea feed predominantly on non-segmented coelomate worms in mud-sand areas, and bivalves on sandy substrate [47]. RKC diets also appear to shift with prey availability [49]; thus RKC diets likely shift from a clam-based diet when they are living on sand in cold years, to a more worm-based diet when living over finer sediments in warm years. The energetic consequences of this suspected diet shift warrant further investigation, as well as potential competitive interactions with other predators utilizing the same prey base.

Management implications

Given the differences in crab distribution with temperature regime, observed both in summer (NMFS trawl survey) and autumn (DFL data), it is important to evaluate the effectiveness of fixed closure areas designed to protect crab from trawl fisheries. The RKCSA and NBBTCA were closed to trawling to protect adult crab and juveniles, respectively [8], yet limited data were available to guide the placement of these closures. The NBBTCA was placed

in an area known to meet juvenile habitat requirements [50], while the RKCSA was created based on crab abundance from summer trawl surveys and high bycatch of RKC in a few seasons for some of the winter flatfish fisheries [8]. Despite the limited data to support these decisions, in the years directly after implementation of the RKCSA, bycatch rates declined [51] and bycatch has since remained well below the prohibited species catch [20,52]. However, while trawl closures do have clear benefits, the displacement of trawling effort to other locations may cause additional concerns, including increased Pacific halibut (*Hippoglossus stenolepis*) bycatch [51]. These potential negative impacts have motivated an interest in evaluating the effectiveness of trawl closure areas in protecting Bristol Bay RKC.

DFLs yield distribution information in the autumn, just before the start of winter trawl fisheries. Such information should be particularly relevant in evaluating closure areas, relative to the summer survey data. In warm years, 60% of legal male crab were caught by the crab fishery in the RKCSA, while less than 25% were caught there in cold years (Fig. 4.8). In most cold years, over 50% of crab were caught in the NBBTCA. In some cold years, crab also occurred in trawlable waters, both in the RKCSS and in other areas of Zone 1 (Fig. 4.1). These data indicate that crab were caught in high numbers in the closure areas. However, this information describes the distribution of crab during the autumn crab fishery, whereas trawl closures constrain the flatfish fisheries that mostly take place in the winter. If DFL data are to inform the placement of winter trawl closures, additional study is needed to determine the relationship between autumn and winter crab distributions. Toward this end, a few years of fishery independent surveys during autumn and winter would be invaluable.

Conclusions

DFLs can help elucidate the distribution of Bristol Bay RKC during the autumn, when standard survey data are not available. They provide insights into patterns in crab distribution with temperature regime and can help inform the placement of closure areas to achieve crab conservation objectives. DFLs provide accurate spatial data, given the similarity of results derived from observer and DFL data. Given the many uses of DFL data, it is essential that they become more accessible in the future for other Bering Sea and Aleutian Island crab fisheries (e.g., golden king crab [*Lithodes aequispinus*] crab, Tanner crab [*Chionoecetes bairdi*], snow crab [*Chionoecetes opilio*]). The Bristol Bay RKC fleet has begun to transition from paper to electronic logbooks, which should make these data more readily available to managers and avoid additional human transcriber errors. RKC are highly mobile species, thus the summer survey cannot fully explain their distribution; DFLs are an important tool that can supplement summer surveys, helping to improve fishery management decisions that concern crab distribution.

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thus all analyses and interpretations are the responsibility of the authors. We greatly appreciate the help of Ian Zacher, Cortney Bue, Jonah Yakunin, Yorise Yakunin, and Diego Madrid in digitizing DFLs. We thank Ginny Eckert, Frank Morado, and Lara Horstmann for aiding with project design and review.

Data Accessibility Statement

Under federal law, fisheries data on individual fishers is confidential (50 CFR 229.11). Thus, daily fishing log (DFL) and observer data, which contain information on fishing locations and catch of individual vessels, are confidential and cannot be made publicly available. These data are stored at the Alaska Department of Fish and Game (ADF&G) office in Dutch Harbor, Alaska. To access data contact Miranda Westphal (miranda.westphal@alaska.gov). However, anyone wishing to access these data will have to obtain a confidentiality agreement with ADF&G, which is at the discretion of ADF&G.

References

1. Blackford MG. Pioneering a modern small business: Wakefield Seafoods and the Alaskan frontier. Industrial development and the social fabric, Vol. 6. Bingley: Emerald Group Publishing Limited; 1979.
2. Kruse GH, Zheng J, Stram DL. Recovery of the Bristol Bay stock of red king crabs under a rebuilding plan. ICES J Mar Sci. 2010;67: 1866–1874. doi:10.1093/icesjms/fsq136
3. Loher T, Armstrong DA. Historical changes in the abundance and distribution of ovigerous red king crabs (*Paralithodes camtschaticus*) in Bristol Bay (Alaska), and potential relationship with bottom temperature. Fish Oceanogr. 2005;14: 292–306. doi:10.1111/j.1365-2419.2005.00337.x

4. Garber-Yonts B, Lee J. Economic status report summary: BSAI crab fisheries, 2016 [Internet]. National Marine Fisheries Service. Seattle, Washington; 2016 Sept [cited 2018 Feb 19]. Available from: <http://www.npfmc.org/wp-content/PDFdocuments/resources/SAFE/CrabSAFE/2016CrabEconomicSAFEappendix.pdf>
5. Fina M. Rationalization of the Bering Sea and Aleutian Islands crab fisheries. Mar Policy. 2005;29: 311–322. doi:10.1016/j.marpol.2004.05.005
6. Amendment 18: Fishery management plan for Bering Sea/ Aleutian Islands king and Tanner crabs [Internet]. North Pacific Fishery Management Council. Anchorage, Alaska; 2004 Jun [cited 2018 Feb 19]. Available from: <https://alaskafisheries.noaa.gov/sites/default/files/ktc18fmp.pdf>
7. 2014-2015 Commercial king and Tanner crab fishing regulations. Alaska Department of Fish and Game. Juneau, Alaska; 2014.
8. Amendment 37: Fishery management plan for the groundfish fishery of the Bering Sea and Aleutian Islands [Internet]. North Pacific Fishery Management Council. Anchorage, AK; 1996 [cited 2018 Feb 20]. Available from: <https://alaskafisheries.noaa.gov/sites/default/files/bsaiamd37fmp.pdf>
9. Bright DB. Life histories of the king crab, *Paralithodes camtschatica*, and the “Tanner” crab, *Chionoecetes bairdi*, in Cook Inlet, Alaska. Ph.D. Dissertation, University of Southern California. 1967.

10. Chilton EA, Foy RJ, Armistead CE. Temperature effects on assessment of red king crab in Bristol Bay, Alaska. In: Kruse GH, Eckert GL, Foy RJ, Lipcius RN, Sainte-Marie B, Stram DL, et al., editors. Biology and management of exploited crab populations under climate change. Fairbanks: Alaska Sea Grant College Program, University of Alaska Fairbanks, AK-SG-10-01; 2010. pp. 249–263. doi:<https://10.4027/bmespcc.2010>
11. Dew CB. Red king crab mating success, sex ratio, spatial distribution, and abundance estimates as artifacts of survey timing in Bristol Bay, Alaska. N Am J Fish Manag. 2008;28: 1618–1637. doi:10.1577/M07-038.1
12. Loher T. Recruitment variability in southeast Bering Sea red king crab (*Paralithodes camtschaticus*): The roles of early juvenile habitat requirements, spatial population structure, and physical forcing mechanisms. Ph.D. Dissertation, University of Washington. 2001.
13. Mueter FJ, Litzow MA. Sea ice retreat alters the biogeography of the Bering Sea continental shelf. Ecol Appl. 2008;18: 309–320. doi:10.1890/07-0564.1
14. Stabeno PJ, Kachel NB, Moore SE, Napp JM, Sigler M, Yamaguchi A, et al. Comparison of warm and cold years on the southeastern Bering Sea shelf and some implications for the ecosystem. Deep Sea Res Part 2 Top Stud Oceanogr. 2012;65–70: 31–45. doi:10.1016/j.dsr2.2012.02.020
15. Duffy-Anderson JT, Stabeno PJ, Siddon EC, Andrews AG, Cooper DW, Eisner LB, et al. Return of warm conditions in the southeastern Bering Sea: Phytoplankton - fish. PLOS ONE. 2017;12: e0178955. Available: <https://doi.org/10.1371/journal.pone.0178955>

16. Evans D, Fey M, Foy R, Olson J. The evaluation of adverse impacts from fishing on crab essential fish habitat: NMFS and NPFMC staff discussion paper, Item C-4(c)(1) [Internet]. North Pacific Fishery Management Council. Anchorage, Alaska; 2012 Jan [cited 2018 Feb 20]. Available from: https://www.npfmc.org/wp-content/PDFdocuments/conservation_issues/EFH/BBRKC_EFH212.pdf
17. Daly B, Armistead C, Foy R. The 2016 eastern Bering Sea continental shelf bottom trawl survey: Results for commercial crab species [Internet]. U.S. Department of Commerce, NOAA Technical Memorandum. NMFS - AFSC-327. 2016 Sept [cited 2018 Feb 20]. doi:10.7289/V5/TM-AFSC-327
18. Fitch H, Schwenzfeier M, Baechler B, Hartill T, Salmon M, Deiman M, et al. Annual management report for the commercial and subsistence shellfish fisheries of the Aleutian Islands, Bering Sea and the westward region's shellfish observer program, 2011/12 [Internet]. Alaska Department of Fish and Game, Fishery Management Report No. 12-22. Anchorage, Alaska; 2014 May [cited 2018 Feb 20]. Available from: <http://www.adfg.alaska.gov/FedAidPDFs/FMR14-54.pdf>
19. Aydin K, Barbeaux S, Barnard D, Chilton L, Conners C, Dalton M, et al. Stock assessment and fishery evaluation report for the groundfish resources of the Bering Sea/ Aleutian Islands regions [Internet]. North Pacific Fishery Management Council. Anchorage, Alaska; 2016 Sept [cited 2018 Feb 20]. Available from: <https://www.afsc.noaa.gov/REFM/Docs/2016/BSAIsafe.php>

20. Bristol Bay red king crab essential fish habitat and bycatch interactions with groundfish fisheries, Item C-1(b) [Internet]. North Pacific Fishery Management Council. 2013 Jan [cited 2018 Feb 20]. Available from: https://www.npfmc.org/wp-content/PDFdocuments/conservation_issues/EFH/BBRKC_EFH213.pdf
21. Bush K, Dorn M, Eckert G, Foy R, Garber-Yonts B, Hamazaki T, et al. Stock assessment and fishery evaluation report for the king and Tanner crab fisheries of the Bering Sea and Aleutian Islands regions [Internet]. North Pacific Fishery Management Council. Anchorage, Alaska; 2016 Sept [cited 2018 Feb 20]. Available from: http://www.npfmc.org/wp-content/PDFdocuments/resources/SAFE/CrabSAFE/2016CrabSAFE_final.pdf
22. Nelson TA, Boots B. Detecting spatial hot spots in landscape ecology. *Ecography* (Cop). 2008;31: 556–566. doi:10.1111/j.0906-7590.2008.05548.x
23. Boots B. Local measures of spatial association. *Écoscience*. 2002;9: 168–176. doi:10.1080/11956860.2002.11682703
24. O’Sullivan D, Unwin D. *Geographic information analysis*, 2nd edition. Hoboken: John Wiley & Sons; 2010.
25. Ord JK, Getis A. Local spatial autocorrelation statistics: Distributional issues and an application. *Geogr Anal*. 2010;27: 286–306. doi:10.1111/j.1538-4632.1995.tb00912.x
26. Moran PAP. Notes on continuous stochastic phenomena. *Biometrika*. 1950;37: 17–23. doi:10.2307/2332142
27. Anselin L. Local indicators of spatial association-LISA. *Geogr Anal*. 2010;27: 93–115. doi:10.1111/j.1538-4632.1995.tb00338.x

28. Ord JK, Getis A. Testing for local spatial autocorrelation in the presence of global autocorrelation. *J Reg Sci.* 2001;41: 411–432. doi:10.1111/0022-4146.00224
29. Anselin L, Syabri I, Kho Y. GeoDa: An introduction to spatial data analysis. *Geogr Anal.* 2006;38: 5–22. doi:10.1111/j.0016-7363.2005.00671.x
30. Anselin L. GeoDa™ 0.9 user's guide [Internet]. Center for Spatially Integrated Social Science. 2003 Jun [cited 2018 Feb 20]. Available from:
<http://www.unc.edu/~emch/gisph/geoda093.pdf>
31. ArcGIS Desktop: Release 10.3 [software]. Environmental Systems Research Institute. 2014 Dec [cited 2018 Feb 20]. Available from: <https://www.esri.com/en-us/home>
32. Hsu C. Spatial and temporal distribution patterns of female red king crabs in the southeastern Bering Sea. Ph.D. Dissertation, University of Washington. 1987.
33. Takeshita K, Fujita H, Matsuura S. A note on population structure in the eastern Bering Sea adult red king crab, *Paralithodes camtschatica*. In: Lowell Wakefield Fisheries Symposium. Proceedings of the International Symposium on King and Tanner Crabs. 1989 Nov 28-30; Anchorage, Alaska. Fairbanks: Alaska Sea Grant College Program (AK-SG-90-04); 1990. pp. 427–433. Available from: ftp://ftp.library.noaa.gov/noaa_documents.lib/Sea_Grant_documents/Alaska/Report_90-04.pdf
34. Shirley TC, Shirley SM, Korn S. Incubation period, molting, and growth of female red king crabs: Effects of temperature. In: Lowell Wakefield Fisheries Symposium. Proceedings of the International Symposium on King and Tanner Crabs. 1989 Nov 28-30; Anchorage, Alaska. Fairbanks: Alaska Sea Grant College Program (AK-SG-90-04); 1990. pp. 51–63. Available from: ftp://ftp.library.noaa.gov/noaa_documents.lib/Sea_Grant_documents/Alaska/Report_90-04.pdf

35. Otto R., Macintosh RA, Cummiskey PA. Fecundity and other reproductive parameters of female red king crab (*Paralithodes camtschatica*) in Bristol Bay and Norton Sound, Alaska. In: Lowell Wakefield Fisheries Symposium. Proceedings of the International Symposium on King and Tanner Crabs. 1989 Nov 28-30; Anchorage, Alaska. Fairbanks: Alaska Sea Grant College Program (AK-SG-90-04); 1990. pp. 65–90. Available from: ftp://ftp.library.noaa.gov/noaa_documents.lib/Sea_Grant_documents/Alaska/Report_90-04.pdf
36. Zheng J, Kruse GH. Recruitment variation of eastern Bering Sea crabs: Climate-forcing or top-down effects? Prog Oceanogr. 2006;68: 184–204. doi:10.1016/j.neulet.2005.10.035
37. Stone RP, Oclair CE, Shirley TC. Aggregating behavior of ovigerous female red king crab, *Paralithodes camtschaticus*, in Auke Bay, Alaska. Can J Fish Aquat Sci. 1993;50: 750–758.
38. Simpson RR, Shippen HH. Movement and recovery of tagged king crabs in the eastern Bering Sea, 1955-63. Bull No 24, Int North Pacific Fish Comm. 1968;24: 111–123. Available from: [http://www.npafc.org/new/inpfc/INPFC Bulletin/Bull No.24/Bull24 p111-123 \(Simpson + Shippen\).pdf](http://www.npafc.org/new/inpfc/INPFC_Bulletin/Bull_No.24/Bull24_p111-123_(Simpson_+Shippen).pdf)
39. Nippes WE. Westward region shellfish report to the Alaska Board of Fisheries [Internet]. Alaska Department of Fish and Game, Regional Information Report No. 4K91-4. Kodiak, Alaska; 1991 Mar [cited 2018 Feb 20]. Available from: <http://www.adfg.alaska.gov/fedaids/rir.4k.1991.04.pdf>

40. ADF&G staff. Annual management report for the shellfish fisheries of the westward region, 1999 [Internet]. Alaska Department of Fish and Game, Reg Inf Rep No 4K00-55. Kodiak, Alaska; 2000 July [cited 2018 Feb 20]. Available from:
<http://www.adfg.alaska.gov/FedAidPDFs/RIR.4K.2000.55.pdf>
41. Stevens BG, Lovrich GA. Chapter 1: King crabs of the world: Species and distributions. In: Stevens BG, editor. King crabs of the world, biology and fisheries management. Boca Raton: CRC Press; 2014. pp. 1–30.
42. Yeung C, McConnaughey RA. Community structure of eastern Bering Sea epibenthic invertebrates from summer bottom-trawl surveys 1982 to 2002. *Mar Ecol Prog Ser*. 2006;318: 47–62. doi:10.3354/meps318047
43. Christiansen JS, Sparboe M, Sæther BS, Siikavuopio SI. Thermal behaviour and the prospect spread of an invasive benthic top predator onto the Euro-Arctic shelves. *Divers Distrib*. 2015;21: 1004–1013. doi:10.1111/ddi.12321
44. Smith KR, McConnaughey RA. Surficial sediments of the eastern Bering Sea continental shelf: EBSSSED database documentation [Internet]. U.S. Department of Commerce, NOAA Technical Memorandum NMFS-AFSC-104. 1999 [cited 2018 Feb 20]. Available from: <https://www.afsc.noaa.gov/Publications/AFSC-TM/NOAA-TM-AFSC-104.pdf>
45. McGonigle C, Collier JS. Interlinking backscatter, grain size and benthic community structure. *Estuar Coast Shelf Sci*. 2014;147: 123–136. doi:10.1016/j.ecss.2014.05.025
46. Wieser W. The effect of grain size on the distribution of small invertebrates inhabiting the beaches of Puget Sound. *Limnol Oceanogr*. 1959;4: 181–194.
doi:10.4319/lo.1959.4.2.0181

47. McConnaughey RA, Smith KR. Associations between flatfish abundance and surficial sediments in the eastern Bering Sea. *Can J Fish Aquat Sci.* 2000;57: 2410–2419.
doi:10.1139/f00-219
48. Falk-Petersen J, Renaud P, Anisimova N. Establishment and ecosystem effects of the alien invasive red king crab (*Paralithodes camtschaticus*) in the Barents Sea - A review. *ICES J Mar Sci.* 2011;68: 479–488. doi:10.1093/icesjms/fsq192
49. Sundet JH, Rafter E., Nilssen EM. Sex and seasonal variation in the stomach content of red king crab, *Paralithodes camtschaticus* in the southern Barents Sea. In: Klein JCVV, Schram FR, editors. The biodiversity crisis and Crustacea: Proceedings of the fourth international crustacean congress, Volume 2. 1998 July 20-24; Amsterdam, Netherlands. Amsterdam: A.A. Balkema Publishers; 2000. pp. 193–200.
50. Ackley D, Witherell D. Development of a marine habit protection area in Bristol Bay, Alaska. In: Lowell Wakefield Fisheries Symposium. Ecosystem Approaches for Fisheries Management. 1998 Sept 30 - Oct 3; Anchorage, Alaska. Fairbanks: University of Alaska Sea Grant College Program (AK-SG-99-01); 1999. pp. 511–526.
doi:10.4027/eafm.1999.38
51. Abbott JK, Haynie AC. What are we protecting? Fisher behavior and the unintended consequences of spatial closures as a fishery management tool. *Ecol Appl.* 2012;22: 762–777. doi:10.1890/11-1319.1

52. Fissel B, Dalton M, Felthoven R, Garber-Yonts B, Haynie A, Kasperski S, et al. Stock assessment and fishery evaluation report for the groundfish fisheries of the Gulf of Alaska and Bering Sea/ Aleutian Islands area: Economic status of the groundfish fisheries off Alaska, 2015 [Internet]. North Pacific Fishery Management Council. Seattle, Washington; 2016 Dec [cited 2018 Feb 20]. Available from: <https://www.afsc.noaa.gov/REFM/Docs/2016/economic.pdf>

Appendices

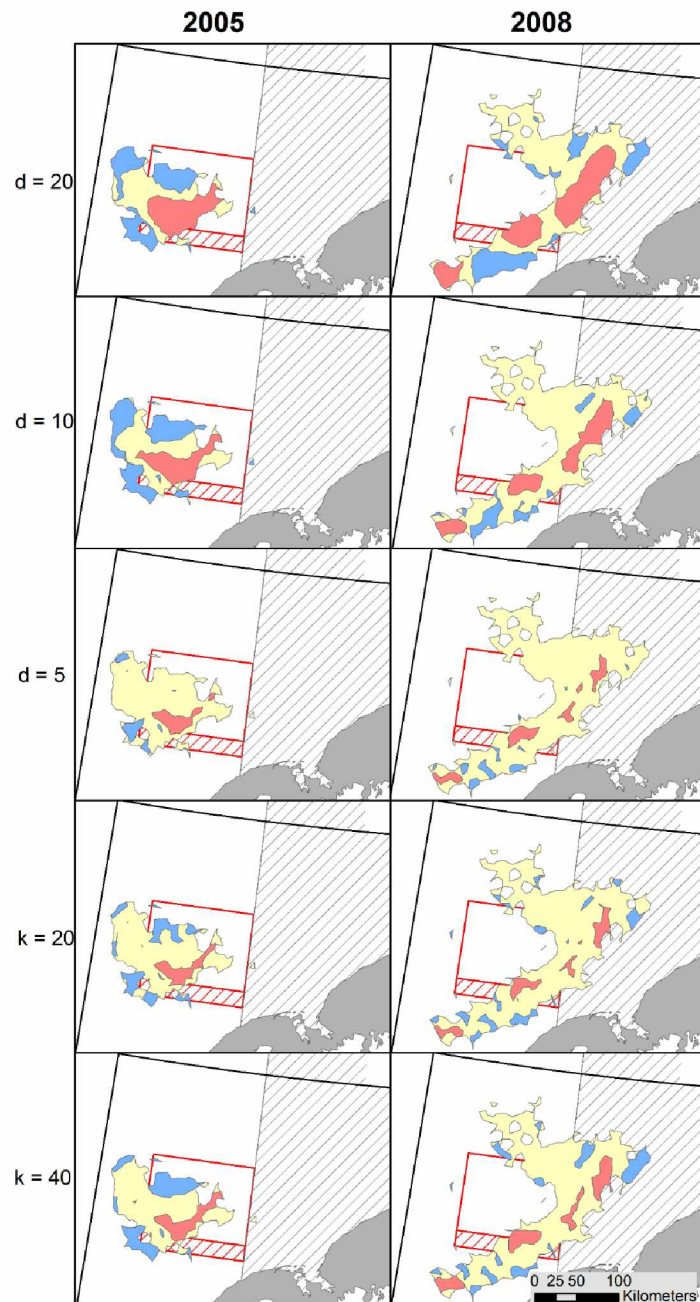


Figure 4.1 Sup. Comparisons of weighting methods in Getis-Ord hot spot, G_i^* analyses. Analyses performed on catch per unit effort data from daily fishing logs in the Bristol Bay red king crab fishery. Analyses were conducted using 5 weighting methods: a distance band (d) of 20, 10, and 5 km and a neighbor number (k) of 20 and 40. Results shown for two sample years, 2005 and 2008. Areas with trawling restrictions are outlined in red or gray as described in Fig. 4.1.

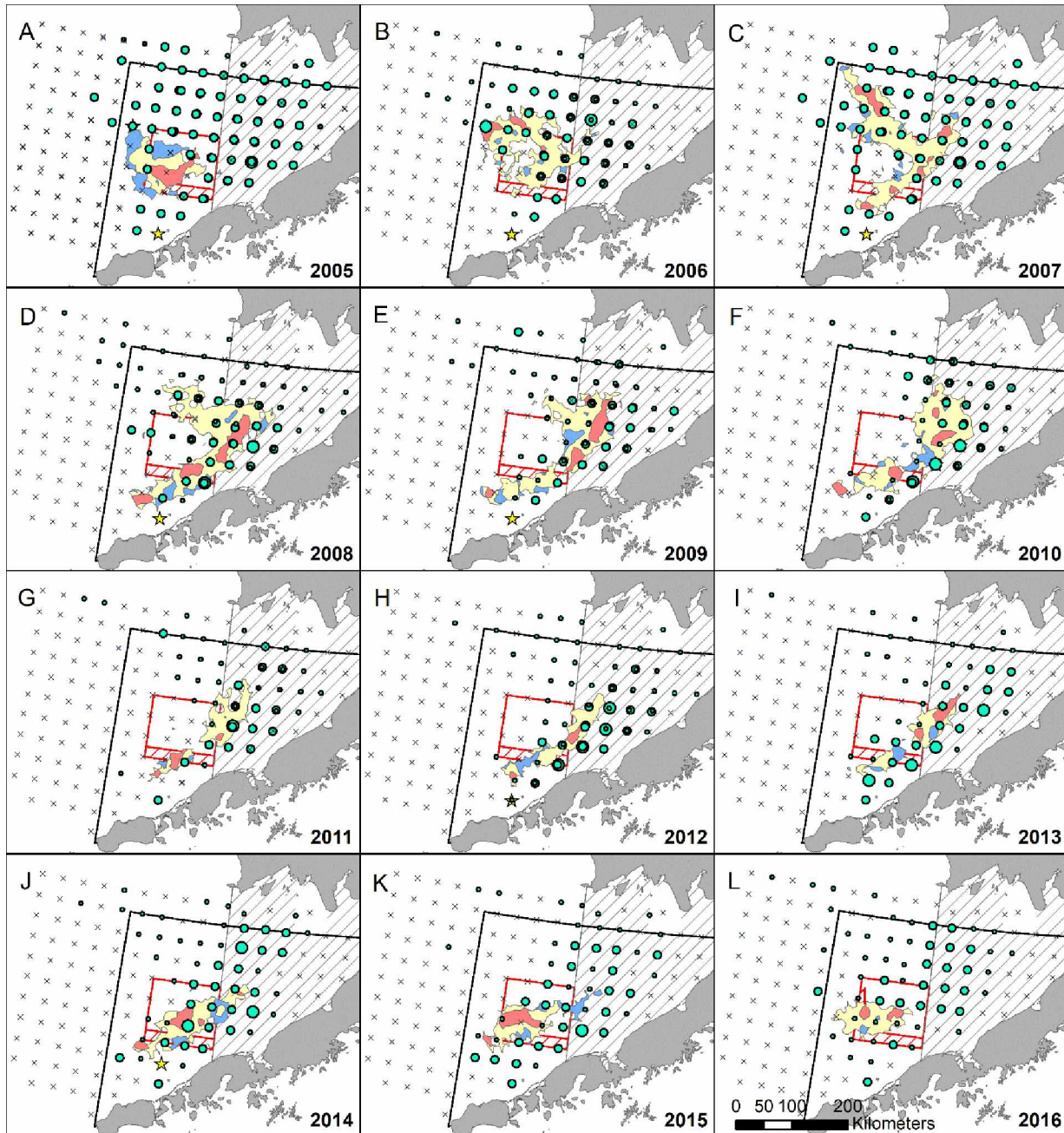


Figure 4.2 Sup. Autumn versus summer distribution of Bristol Bay red king crab over 2005 – 2016. Summer data are from the National Marine Fisheries Service (NMFS) trawl survey and winter data are from daily fishing logs (this study, Fig. 4.3). Both datasets show the catch of legal sized male crab only. NMFS trawls are on a grid system, with crab caught per km²: x = no catch, small circle = 1 – 100 crab, medium circle = 101 – 500 crab, large circle = 501 – 1,000 crab, and yellow star = >1,000 crab. A Getis-Ord hot spot analysis was performed on daily fishing log data: red = statistically significant ($\alpha < 0.01$) high catch per unit effort (CPUE), blue = statistically significant low CPUE, yellow = not statistically different than the mean, and white = areas where no fishing occurred. Areas with restrictions on trawling are outlined in red or gray and described in Figure 4.1.

CHAPTER 5: CONCLUSIONS

The large, commercially harvested lithodid crab (Family Lithodidae) are collectively referred to as king crab (Otto, 2014). Lithodid crab biogeography and evolutionary history are tied to temperature, and because of temperature tolerances in the larval phase, these crab are restricted to habitats with water temperatures between 0 and 15 °C (Shirley & Shirley, 1989; Hall & Thatje, 2009). As a result, it is only at high latitudes (North Pacific and around the southern tip of South America) that lithodids can live in shallow waters (< 200 m); where as in more temperate and tropical waters lithodids are restricted to depths below 200 m (Hall & Thatje, 2009). Extremely cold waters (< 0 °C) also prevent lithodids from inhabiting the high Arctic and the Antarctic shelves, although this may be changing as temperatures increase (Smith *et al.*, 2012), and introduced king crab are thriving in the Barents Sea (Falk-Petersen *et al.*, 2011). The temperature restrictions on king crab, coupled with intensive fishing pressures, and changes in climate, make managing these species challenging, especially when there is limited information on their biology and interactions with other ecosystem components.

The primary objective of the research described here was to contribute to our understanding of Alaskan king crab biology and ecology, by investigating how king crab distributions change with temperature regimes and explore the biology and environmental tolerances of an important king crab parasite. These studies may contribute to more informed management in a changing climate.

Larval Biology of *Briarosaccus*

Rhizocephalan barnacles are parasitic castrators that primarily infect decapod crustaceans (Høeg, 1995). Members of the genus *Briarosaccus* infect many lithodid species throughout the world (e.g., Hawkes *et al.*, 1986; Abello & Macpherson, 1992; Guzman *et al.*, 2002), with *B.*

regalis infecting red and blue king crab (*Paralithodes camtschaticus* and *P. platypus*, respectively) and *B. auratum* infecting golden king crab (*Lithodes aequispinus*) in Alaska (Noever *et al.*, 2016). Prevalence of *B. regalis* generally appears low in red king crab (<1%; Sloan, 1985; Hawkes *et al.*, 1986; unpubl. ADF&G survey and observer program data), although higher prevalence has occurred in blue king crab, especially in isolated fjords and bays (e.g., 76% infected in Glacier Bay in 1984; Hawkes *et al.*, 1986). If the prevalence of *B. regalis* were to increase, it could lower king crab fecundity, with the potential for future stock declines.

To better understand how environmental factors in Alaska may influence *B. regalis* prevalence, I studied the effects of temperature and salinity on the larval stage. Nauplius larvae were reared at 7 temperature (2 to 16 °C) and 8 salinity (19 to 40) treatments to determine larval survival and development rates. Maximum survival occurred from 4 to 12 °C and at salinities between 25 and 34. Currently, bottom water temperatures around Alaska are generally on the colder end of what *B. regalis* larvae tolerated in this study, ranging from ≤ -1 to 8 °C (Stone *et al.*, 1992, 1993; Stabeno *et al.*, 2007), with king crab typically avoiding water ≤ 2 °C (Chilton *et al.*, 2010). Based on my results, these temperatures would promote high *B. regalis* larval survival, albeit with relatively slow development. *B. regalis* appears well adapted to deal with future increases in temperature (Royer & Grosch, 2006; Wang *et al.*, 2012), and changes in freshwater runoff and salinity (Jansson *et al.*, 2003; Royer & Grosch, 2006), because it tolerates temperatures and salinities beyond the range that it currently experiences.

Future work

To further understand how *B. regalis* prevalence will change under future climate scenarios, additional studies should focus on other aspects of the life cycle, particularly the process of infection. We also lack an understanding of interacting effects of temperature and

salinity, as well as other potential environmental stressors (e.g., pH, oxygen saturation), on both the host and the parasite that would allow us to fully grasp how changing conditions will impact this host-parasite relationship.

Briarosaccus regalis larvae had high survival across relatively wide temperature and salinity ranges, given that host crab live in deep waters with comparatively low variability. However, it is unknown whether *B. regalis* larvae *in situ* remain at depth where they are released or migrate into surface waters that can be warmer and fresher. The former appears more probable, given that they are lecithotrophic, and like other members of the Peltogastridae, lack a naupliar eye that is used for phototaxis in other rhizocephalan families (Kashenko & Korn, 2003). Future studies should determine the vertical position and geographical distribution of *B. regalis* larvae throughout development (e.g., early naupliar stages, late naupliar stages, and cyprid stage). Repeated plankton tows taken at different depths in the water column would be ideal, but unless prevalence of *B. regalis* is found to be very high in a constrained area (e.g., a fjord), much effort could yield very few or no larvae, given the low prevalence of the parasite. Instead quantitative PCR could be performed on water samples across different depths. In addition, the behavior of larvae could be observed when placed at different heights in an artificial water column (e.g., Schmalenbach & Buchholz, 2010). These results could elucidate, not only how environmental factors that vary with depth might affect larvae, but also the likely level of larval dispersal and retention.

Metabolites and Infection

Rhizocephalans not only castrate their hosts, they can cause changes in host morphology, physiology, and behavior. Males are generally feminized, adopting female egg-caring behaviors and becoming morphologically more similar to females (Høeg, 1995). Infected crab tend to the

externa, cleaning and aerating the eggs (Ritchie & Høeg, 1981; deVries *et al.*, 1989). Despite the morphological and behavioral changes in host crab, the underlying pathophysiology and mechanisms of “host control” are poorly understood.

To better understand the physiological processes involved with rhizocephalan infection, I used an environmental metabolomics approach (Lankadurai *et al.*, 2013) to investigate the effects of rhizocephalan infection on the crab metabolome using red and golden king crab tissues. I compared the metabolite profiles (e.g., signaling molecules, hormones) of king crab with and without rhizocephalan infections. Hundreds of putative metabolites were identified, yet few differed with crab sex and no metabolites could differentiate infected and healthy crab (regardless of crab sex). Infection year, plus local and regional scale locations, caused large changes in the crab metabolome, which likely masked differences between sex and infection status.

Future work

There are several different methods that could increase detection of metabolites that distinguish healthy and infected crab. Most other liquid chromatography mass spectrometry (LC-MS)-based metabolomics studies with crustaceans have been conducted under controlled laboratory conditions (Ralston-Hooper *et al.*, 2011; Maity *et al.*, 2012, 2013; Laith *et al.*, 2017). Theoretically, this would help to stabilize the metabolome, increasing the detectability of infection state; however, laboratory stabilization does not always decrease variability in the metabolome of invertebrates (Alvarez *et al.*, 1995). Most metabolomics studies investigating the effects of stressors on crustaceans have used nuclear magnetic resonance (NMR)-based metabolomics (Schock *et al.*, 2010; Hammer *et al.*, 2012). NMR has higher precision and accuracy in metabolite identification, but LC-MS has a higher sensitivity for rare metabolites

(Beltran *et al.*, 2012). NMR metabolomics could help elucidate physiological changes in infected crab that lead to changes in common metabolites (e.g., glucose, lactate, amino acids). It has been hypothesized that rhizocephalan interna may release a “diffusible substance” to control crab hosts (Rubiliani, 1985), yet if this substance is at a low concentration NMR methods would likely not identify it and LC-MS methods would be needed. Future studies could also use targeted metabolomics to focus on specific compounds that are likely to change with rhizocephalan infection, like those involved with immune response, molting, reproduction, and growth. For example, much of the innate immune response of crustaceans is involved in the complex prophenoloxidase activating system (proPO system) (Smith *et al.*, 2003; Vazquez *et al.*, 2009). Precursors and products in the proPO system could be targeted to reveal the immune response of infected crab.

To truly understand how a rhizocephalan manipulates its crab host and the host’s response to infection, metabolites should be measured repeatedly throughout the infection process, from cyprid settlement, through the interna phase, and ultimately to the production of the externa. *Briarosaccus* and its king crab hosts would not be ideal for this type of study, because these large crab are difficult to keep in a laboratory in large numbers and the infection process has never been studied for this species. One of the smaller, better studied rhizocephalans, with a relatively quick internal phase (time from infection to emergence of externa) would be more appropriate for this type of study (e.g., *Sacculina carcini* or *Lernaeodiscus porcellanae*). After the changes in metabolites during the infection process are understood, a focused study could then be conducted with the more commercially important king crab and *Briarosaccus*.

Log Books and Crab Distribution

Summer distributions of Bristol Bay red king crab are well documented from surveys, but at other times of the year, their distribution patterns are poorly understood. Most king crab bycatch occurs in winter groundfish trawl fisheries (Evans *et al.*, 2012; Aydin *et al.*, 2016), and there are areas closed to trawling, specifically to protect king crab. However, lack of detailed data on winter crab distributions makes evaluating the effectiveness of these closures difficult. Daily fishing logs, kept by skippers in the red king crab fleet since 2005, contain detailed information on the spatial distribution of catch and effort of legal sized male crab during the autumn crab fishery. However, data contained in these hand-written logbooks have not been readily accessible.

Daily fishing logs from 2005 to 2016 were digitized and I used spatial information to infer geographic distributions. These distributions were compared across large-scale temperature regimes in the Bering Sea. In warm years (2005, 2014 – 2016), crab aggregated in the center of Bristol Bay, while in cold years (2007 – 2013), they were closer to the Alaska Peninsula. As temperatures continue to shift in the Bering Sea, it will be important to continue monitoring crab distributions outside the summer survey period.

Future work

Fishermen's logbooks provided information on how crab used two trawl closure areas in Bristol Bay in the autumn, and their relative importance during warm and cold temperature regimes. However, these data describe the distribution of crab during the autumn crab fishery, whereas trawl closures constrain the flatfish fisheries that mostly take place in the winter. If fishing log data are to inform the placement of winter trawl closures, additional study is needed to determine the relationship between autumn and winter crab distributions in Bristol Bay. To

this end, trawl surveys should be conducted during the autumn and winter and in both warm and cold temperature regimes.

I found very distinct distributions of Bristol Bay red king crab under warm and cold temperature regimes; however, the underlying variables driving these changes in distribution are poorly understood. During October and November, when this crab fishery occurs, bottom water temperatures in Bristol Bay are the warmest that crab experience within a year due to mixing of surface waters (Stabeno *et al.*, 2012). There is currently no record of bottom water temperatures across the eastern Bering Sea shelf in the autumn, so it is difficult to evaluate whether distribution patterns are directly related to bottom water temperatures. Future work should focus on recording bottom water temperatures in the autumn, especially in areas that are crab hot spots in both warm and cold years. In conjunction with temperature data, a fine scale comparison of the benthic habitats in the warm and cold year crab hotspots could help elucidate why crab consistently return to these particular areas.

Final Thoughts

The future for king crab populations and fisheries in Alaska is still uncertain. King crab populations are highly dynamic, and it is difficult to understand the factors that contribute to these population fluctuations. The 10-fold decline in the Bristol Bay red king crab stock in the early 1980s is still poorly understood, potentially including over-exploitation, bycatch in trawl fisheries, mismatch in larval release and plankton blooms, low recruitment, increased predation, and parasites (Stevens, 2014). In addition to all of these historical factors that can influence king crab populations, we are now adding new ones, such as climate change and ocean acidification. For instance, waters with lower pH can decrease the survival of juvenile (Long *et al.*, 2013b) and larval (Long *et al.* 2013a) red king crab.

Warmer waters are likely to change distribution patterns of many species in the Bering Sea (Mueter & Litzow, 2008). Since the southern Bering Sea has traditionally oscillated between “warm” and “cold” temperature regimes, depending on the southern extent of the cold pool (Stabeno *et al.*, 2012), it provided a unique opportunity to investigate how king crab respond to changes in temperature. I found clear differences in crab distributions in warm and cold years. As the climate warms, there may be fewer and fewer cold years or cold years could become “cool years”, while warm years become “extra warm years”. The changes in distribution that I found between warm and cold years may provide a glimpse of what distributions will look like in the future. Thus far, Bristol Bay king crab do not move north under warmer water conditions (chapter 4 and Mueter & Litzow, 2008), but instead crab cluster in a fairly narrow region (Fig. 4.3). In 2016, an exceptionally warm year, catch per unit effort in this key region (see hotspots in Fig. 4.3L) was high, even though crab stock levels were low, indicating the importance of this region. It may become necessary to establish this area as a Marine Protected Area. This region is already within a no trawl zone, but it could be important to protect it from the target pot fishery, if it provides unique, essential crab habitat, and acts as a refuge in warm years.

Parasites and disease have been suggested as one of the contributing factors in the Bristol Bay red king crab stock collapse, yet this is largely speculative because a thorough survey for diseases was not conducted until immediately after the population collapsed (Sparks & Morado, 1985). *Briarosaccus* prevalence has the potential to be high, at least in localized king crab populations (e.g., 76% blue king crab in fjords of Glacier Bay; Hawkes *et al.*, 1986), thus this parasite can certainly be an important ecosystem component. I found that *Briarosaccus* larval survival was generally high across a wide range of temperatures. Currently, bottom water

temperatures may sometimes decrease larval survival (when below 4 °C) or at least increase development time. As temperatures increase, there is the potential for *Briarosaccus* survival and infection rates to increase. It will be important to continue monitoring prevalence during surveys and by onboard observers. Since it is unknown how long the internal parasite phase lasts (time between infection and the emergence of the externa), it would be ideal to develop biomarkers in hemolymph that could indicate infection. Metabolites are one possible biomarker. Although I was unable to identify candidate biomarkers, this study lays the groundwork for understanding the variability in the king crab metabolome.

It is my hope that these studies will be built on in the future to more knowledgeably guide the placement of trawl closure areas, predict crab movement with temperature changes, understand the larval biology of *Briarosaccus regalis* and what that could mean with climate change, and lead to a better understanding of the physiology of *Briarosaccus* infection.

References

- Abello, P. and Macpherson, E. 1992. Epibiosis and rhizocephalan infestation patterns in relation to the reproductive biology of *Lithodes ferox* (Filhol, 1885) (Anomura: Lithodidae). *Journal of Crustacean Biology*, **12**: 561–570.
- Alvarez, F., Hines, A.H. and Reaka-Kudla, M.L. 1995. The effects of parasitism by the barnacle *Loxothylacus panopaei* (Gissler) (Cirripedia: Rhizocephala) on growth and survival of the host crab *Rhithropanopeus harrisii* (Gould) (Brachyura: Xanthidae). *Journal of Experimental Marine Biology and Ecology*, **192**: 221–232.

- Aydin, K., Barbeaux, S., Barnard, D., Chilton, L., Conners, C., Dalton, M., Echave, K., Fissel, B. and Furuness, M. 2016. *Stock Assessment and Fishery Evaluation Report for the Groundfish Resources of the Bering Sea/ Aleutian Islands Regions*. North Pacific Fishery Managment Council, Anchorage, Alaska.
- Beltran, A., Suarez, M., Rodríguez, M.A., Vinaixa, M., Samino, S., Arola, L., Correig, X. and Yanes, O. 2012. Assessment of compatibility between extraction methods for NMR- and LC/MS-based metabolomics. *Analytical Chemistry*, **84**: 5838–5844.
- Chilton, E.A., Foy, R.J. and Armistead, C.E. 2010. Temperature effects on assessment of red king crab in Bristol Bay, Alaska. In: *Biology and Management of Exploited Crab Populations under Climate Change* (G. H. Kruse et al., eds), pp. 249–263. Alaska Sea Grant College Program (AK-SG-10-01), Fairbanks.
- deVries, M., Rittschof, D. and Forward, R. 1989. Response by rhizocephalan-parasitized crabs to analogues of crab larval-release pheromones. *Journal of Crustacean Biology*, **9**: 517–524.
- Evans, D., Fey, M., Foy, R. and Olson, J. 2012. *The evaluation of adverse impacts from fishing on crab essential fish habitat: NMFS and NPFMC staff discussion paper, Item C-4(c)(1) January 2012*. North Pacific Fishery Managment Council, Anchorage, Alaska.
- Falk-Petersen, J., Renaud, P. and Anisimova, N. 2011. Establishment and ecosystem effects of the alien invasive red king crab (*Paralithodes camtschaticus*) in the Barents Sea-A review. *ICES Journal of Marine Science*, **68**: 479–488.
- Guzman, G.L., Moreno, R.A. and Moyano, H.I. 2002. Nuevos hospedadores para *Briarosaccus callosus* Boschma, 1930. *Boletin de la Sociedad de Biologia de Concepcion, Chile*, **73**: 83–86.

- Hall, S. and Thatje, S. 2009. Global bottlenecks in the distribution of marine Crustacea: Temperature constraints in the family Lithodidae. *Journal of Biogeography*, **36**: 2125–2135.
- Hammer, K.M., Pedersen, S.A. and Storseth, T.R. 2012. Elevated seawater levels of CO₂ change the metabolic fingerprint of tissues and hemolymph from the green shore crab *Carcinus maenas*. *Comparative Biochemistry and Physiology - Part D: Genomics and Proteomics*, **7**: 292–302.
- Hawkes, C.R., Meyers, T.R. and Shirley, T.C. 1986. Prevalence of the parasitic barnacle *Briarosaccus callosus* on king crabs of southeastern Alaska. *Transactions of the American Fisheries Society*, **115**: 252–257.
- Høeg, J.T. 1995. The biology and the life-cycle of the rhizocephala (Cirripedia). *Journal of the Marine Biological Association of the United Kingdom*, **75**: 517–550.
- Jansson, P., Hock, R. and Schneider, T. 2003. The concept of glacier storage: A review. *Journal of Hydrology* **282**: 116–129.
- Kashenko, S.D. and Korn, O.M. 2003. Combined effects of seawater temperature and salinity on development of the larvae of the rhizocephalan *Peltogaster reticulatus* (Crustacea: Cirripedia). *Russian Journal of Marine Biology*, **29**: 150–155.
- Laith, A.A., Ambak, M., Abol-Munafi, A.B., Nurhafizah, W.W.I. and Najiah, M. 2017. Metabolomic analysis of marine and mud crabs based on antibacterial activity. *Aquaculture Reports*, **7**: 7–15.
- Lankadurai, B.P., Nagato, E.G. and Simpson, M.J. 2013. Environmental metabolomics: an emerging approach to study organism responses to environmental stressors. *Environmental Reviews*, **21**: 180–205.

- Long, W.C., Swiney, K.M. and Foy, R.J. 2013a. Effects of ocean acidification on the embryos and larvae of red king crab, *Paralithodes camtschaticus*. *Marine Pollution Bulletin*, **69**: 38–47.
- Long, W.C., Swiney, K.M., Harris, C., Page, H.N. and Foy, R.J. 2013b. Effects of ocean acidification on juvenile red king crab (*Paralithodes camtschaticus*) and Tanner crab (*Chionoecetes bairdi*) growth, condition, calcification, and survival. *PLOS ONE*, **8**: e60959.
- Maity, S., Jannasch, A., Adamec, J., Gribskov, M., Nalepa, T., Höök, T.O. and Sepúlveda, M.S. 2012. Metabolite profiles in starved *Diporeia* spp. using liquid chromatography - mass spectrometry (LC-MS) based metabolomics. *Journal of Crustacean Biology*, **32**: 239–248.
- Maity, S., Jannasch, A., Adamec, J., Watkins, J.M., Nalepa, T., Höök, T.O. and Sepúlveda, M.S. 2013. Elucidating causes of *Diporeia* decline in the Great Lakes via metabolomics: physiological responses after exposure to different stressors. *Physiological and Biochemical Zoology*, **86**: 213–223.
- Mueter, F.J. and Litzow, M.A. 2008. Sea ice retreat alters the biogeography of the Bering Sea continental shelf. *Ecological Applications*, **18**: 309–320.
- Noever, C., Olson, A. and Glenner, H. 2016. Two new cryptic and sympatric species of the king crab parasite *Briarosaccus* (Cirripedia: Rhizocephala) in the North Pacific. *Zoological Journal of the Linnean Society*, **176**: 3–14.
- Otto, R.. 2014. Chapter 5: History of king crab fisheries with special reference to the North Pacific Ocean: Development, maturity, and senescence. In: *King Crabs of the World: Biology and Fisheries Management* (B. G. Stevens, ed), pp. 81–138. CRC Press, Boca Raton.

- Ralston-Hooper, K.J., Adamec, J., Jannash, A., Mollenhauer, R., Ochoa-Acuña, H. and Sepúlveda, M.S. 2011. Use of GC × GC/TOF-MS and LC/TOF-MS for metabolomic analysis of *hyalella azteca* chronically exposed to atrazine and its primary metabolite, desethylatrazine. *Journal of Applied Toxicology*, **31**: 399–410.
- Ritchie, L. and Høeg, J.T. 1981. The life history of *Lernaodiscus porcellanae* (Cirripedia: Rhizocephala) and co-evolution with its porcellanid hosts. *Journal of Crustacean Biology*, **1**: 334–347.
- Royer, T.C. and Grosch, C.E. 2006. Ocean warming and freshening in the northern Gulf of Alaska. *Geophysical Research Letters*, **33**: 1–6.
- Rubiliani, C. 1985. Response by two species of crabs to a rhizocephalan extract. *Journal of Invertebrate Pathology*, **45**: 304–310.
- Schmalenbach, I. and Buchholz, F. 2010. Vertical positioning and swimming performance of lobster larvae (*Homarus gammarus*) in an artificial water column at Helgoland, North Sea. *Marine Biology Research*, **6**: 89–99.
- Schock, T.B., Stancyk, D.A., Thibodeaux, L., Burnett, K.G., Burnett, L.E., Boroujerdi, A.F.B. and Bearden, D.W. 2010. Metabolomic analysis of Atlantic blue crab, *Callinectes sapidus*, hemolymph following oxidative stress. *Metabolomics*, **6**: 250–262.
- Shirley, T.C. and Shirley, S.M. 1989. Temperature and salinity tolerances and preferences of red king crab larvae. *Marine and Freshwater Behaviour and Physiology*, **16**: 19–30.
- Sloan, N.A. 1985. Life history characteristics of fjord-dwelling golden king crabs *Lithodes aequispina*. *Marine Ecology Progress Series*, **22**: 219–228.

- Smith, C.R., Grange, L.J., Honig, D.L., Naudts, L., Huber, B., Guidi, L. and Domack, E. 2012. A large population of king crabs in Palmer Deep on the west Antarctic Peninsula shelf and potential invasive impacts. *Proceedings of the Royal Society B: Biological Sciences*, **279**: 1017–1026.
- Smith, V.J., Brown, J.H. and Hauton, C. 2003. Immunostimulation in crustaceans: Does it really protect against infection? *Fish and Shellfish Immunology*, **15**: 71–90.
- Sparks, A. and Morado, J. 1985. A preliminary report on the diseases of Alaska king crabs. *Proceedings of the International King Crab Symposium, Alaska Sea Grant Report*. Anchorage, Alaska
- Stabeno, P.J., Bond, N.A. and Salo, S.A. 2007. On the recent warming of the southeastern Bering Sea shelf. *Deep Sea Research Part II: Topical Studies in Oceanography*, **54**: 2599–2618.
- Stabeno, P.J., Kachel, N.B., Moore, S.E., Napp, J.M., Sigler, M., Yamaguchi, A. and Zerbini, A.N. 2012. Comparison of warm and cold years on the southeastern Bering Sea shelf and some implications for the ecosystem. *Deep Sea Research Part II: Topical Studies in Oceanography*, **65–70**: 31–45.
- Stevens, B.G. 2014. Chapter 19: Future of king crabs. In: *King Crabs of the World: Biology and Fisheries Management* (B. G. Stevens, ed), pp. 583–594. CRC Press, Boca Raton.
- Stone, R.P., Oclair, C.E. and Shirley, T.C. 1993. Aggregating behavior of ovigerous female red king crab, *Paralithodes camtschaticus*, in Auke Bay, Alaska. *Canadian Journal of Fisheries and Aquatic Sciences*, **50**: 750–758.
- Stone, R.P., Oclair, C.E. and Shirley, T.C. 1992. Seasonal migration and distribution of female red king crabs in a Southeast Alaskan estuary. *Journal of Crustacean Biology*, **12**: 546–560.

Vazquez, L., Alpuche, J., Maldonado, G., Agundis, C., Pereyra-Morales, A. and Zenteno, E.

2009. Review: Immunity mechanisms in crustaceans. *Innate Immunity*, **15**: 179–188.

Wang, M., Overland, J.E. and Stabeno, P. 2012. Future climate of the Bering and Chukchi Seas projected by global climate models. *Deep Sea Research Part II: Topical Studies in Oceanography*, **65**: 46–57.